STUDIES IN BLOOD PRESSURE AND OBSTRUCTIVE SLEEP APNOEA/ HYPOPNOEA SYNDROME

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Abstract

The obstructive sleep apnoea/ hypopnoea syndrome (OSAHS) is a common disorder, affecting around 2–4 % of the middle-aged population. The deleterious consequences of OSAHS include sleepiness, impaired health status, cognitive function, and driving, and possibly increased cardiovascular and cerebrovascular disease. Treatment of OSAHS improves symptoms, sleepiness, cognition, health status and blood pressure. There are different treatments available for OSAHS, of which the most widely used is CPAP with strong evidence supporting its benefit. There is a strong association between OSAHS and hypertension, based on animal, epidemiological, and interventional studies. The pathogenesis of hypertension in OSAHS is still unclear. Sympathetic activity is increased in OSAHS patients during sleep and wakefulness. However, there are different factors contributing to this increase in sympathetic activity, which may lead to the development of hypertension. In addition, altered endothelial function may have a role.

This thesis aims to explore some of these factors, focusing mainly on baroreflex sensitivity (BRS) in a randomized controlled trial, to test the hypothesis that BRS is impaired in sleep apnoea patients, and might be reversed with one month of CPAP therapy. We performed a blinded placebo controlled crossover trial of the effect of CPAP on BRS in newly diagnosed OSAHS patients. Twenty-nine patients were recruited who had an Epworth Sleepiness Scale (ESS) of more than 10 and an apnoea/hypopnoea index (AHI) of more than 15. A control group of ten healthy subjects was also studied. A non-invasive technique was used to evaluate the BRS, using sequential and spectral analysis of BRS during wakefulness. The study did not

show any significant difference between controls and OSAHS patients in the sequential analysis measure as well as the spectral analysis of BRS. Furthermore, the study did not show any significant difference between CPAP and placebo in terms of an effect on any measure of BRS nor in 24-hour blood pressure in the OSAHS patients. Even an a priori sub-group analysis of desaturating and compliant patients (4% Desaturation Index >10 & CPAP use >3.9 hour/night) showed no effect of CPAP on BRS or blood pressure. Patients improved symptomatically with CPAP (P=0.02). A serious defect with the study was the lack of reproducibility of the measurement of BRS. Furthermore, the relatively small sample size and physiological factors such as respiration may also have affected the results. A more reproducible measurement, larger sample size, and controlling other physiological factors would improve the reliability of any future work. The methods used were investigated by a further study of the reproducibility of the technique of baroreflex measurements, by the sequence and the spectral analysis methods of analysing heart rate and systolic blood pressure. This study revealed that measurements of BRS by analysis of spontaneous variations, particularly the spectral domains, are highly variable over time.

A third study assessed endothelial function, as part of the attempt to understand the pathogenesis of development of hypertension and cardiovascular diseases in OSAHS patients and also the possible contribution of hypoxaemia. Twenty newly diagnosed OSHAS patients were studied but showed no significant variation in endothelial function in relation to the extent of nocturnal hypoxaemia (P>0.1).

Declaration

I declare that I have been the principal investigator in all the studies presented in this thesis and that the content of this thesis is my own work. Various members of the Sleep Department staff, whose contributions have been noted in the Acknowledgements, have assisted me in aspects of these studies. They include Dr Peter Wraith, who wrote the computer programme for the baroreflex analysis, and the Sleep staff, who did the full polysomnography and the overnight CPAP titration. In addition, I should like to mention here the contribution of Dr Jacqueline Faccenda for writing the ethical application of the study of the baroreflex and Dr Melanie Cross, who assisted me in the endothelial function study.

The work was performed in the Sleep Department within the Royal Infirmary of Edinburgh, the Wellcome Trust clinical research facilities in the Western General Hospital and in patients' homes between 2000 and 2004.

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Abbreviations

ABPM	Ambulatory blood pressure monitor
ADR	Adrenaline
ADMA	Asymmetric dimethylarginine
AHI	Apnoea hypopnoea index
AI	Apnoea index
BP	Blood pressure
BRS	Baroreflex sensitivity
CPAP	Continuous positive airway pressure
DBP	Diastolic blood pressure
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electro oculogram
ET-1	Endothelin 1
FOSQ	Functional outcomes of sleep questionnaire
HF	High frequency
HR	Heart rate
HRV	Heart rate variability
LF	Low frequency
MAP	Mean arterial pressure
MAD	Mandibular advancement device
MMO	Mandibular Maxillary Osteotomy
MNSA	Muscle sympathetic nervous activity

MRS	Mandibular repositioning splint
MSLT	Multiple sleep latency test
NO	Nitric oxide
OSA	Obstructive Sleep Apnoea
OSAHS	Sleep apnoea/hypopnoea syndrome
PI	Pulse interval
PSG	Polysomnogram
RRI	R-R intervals
SBP	Systolic blood pressure
S D	Standard Deviation
SDB	Sleep-disordered breathing
SF-36	Short form 36
U3P	Uvulopalatopharyngoplasty
VLF	Very low frequency

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Chapter 1

Sleep-Related Breathing Disorders in Adults

1.1 Introduction

The obstructive sleep apnoea/hypopnoea syndrome (OSAHS) is a disorder characterized by repetitive upper airway collapse during sleep in association with daytime sleepiness that has an estimated prevalence of 2% among middle-aged women and 4% among middle-aged men (Young & Finn 1998a). It was recognized only quite recently, although it has been extensively researched in the last 30 years. OSAHS-related features include excessive daytime sleepiness (Guilleminault, Tilkian, & Dement 1976;Stradling, Barbour, & Glennon 2000), neurocognitive impairment (Engleman et al. 2000), and increased motor vehicle accidents (Engleman, Hirst, & Douglas 1997b;George, Boudreau, & Smiley 1996b;Young et al. 1997). It has also been associated with adverse cardiovascular consequences such as hypertension and impairment of cardiovascular variability (see Chapter 2). Treating OSAHS is very rewarding, for the benefit to the patient is enormous in improving the quality of life as well as preventing the long-term sequelae.

1.2. Historical Background

Perhaps the first description of OSAHS was that of Dionysius, tyrant of Heraclea in the fourth century BC who 'lived in fear of suffocation from fat, and adopted a very curious mode of keeping himself awake' (Kryger 1983). Charles Dickens (Dickens 1837) described a case of obesity with hypersomnolence in Joe, the fat boy in Pickwick Papers. Joe weighed 130 kg and slept most of the time, even during meals, knocking on the door or firing cannons. Joe had dropsy (peripheral oedema) and was slow (possibly cognitive dysfunction) and 'red-faced' (polycythemic) (Douglas

2002a). Burwell et al. (1956) first used the term 'Pickwickian syndrome' when he reported a 51-year old man with a history of somnolence associated with persistent ankle swelling. He weighed 120 kg and had polycythaemia, right heart failure, hypoxaemia and hypercapnia and was noted to have apnoea (Douglas 2002a)

The first detailed description of obstructive sleep apnoea came from Gastaut and co-workers in France in 1965, when they published the first paper on polygraphic studies of the episodic diurnal and nocturnal (hypnic and respiratory) manifestations of the syndrome. In these studies, repetitive episodes of cessation of breathing, designated 'sleep apnoea', were recorded and found to be associated with marked oxygen desaturations. It was thought initially that sleep apnoea occurred only in people with the Pickwickian syndrome. However, Guilleminault (Guilleminault, Tilkian, & Dement 1976) reported in 1976 that sleep apnoea can occur in individuals without these characteristics.

1.3 Definition

The definition of sleep apnoeas and hypopnoeas remains controversial. It is generally acceptable to define apnoea in adults as cessation of breathing from the nose and mouth for more than 10 seconds. The definition of a hypopnoea is more contentious and various definitions are used, including a reduction in tidal volume by > 50 % or flow rate from the baseline plus or minus at least a 4% arterial oxygen desaturations (ASDA 1999). The cessation of breathing can be of three major types:

- **Obstructive:** An episode of 10 seconds or longer when airflow past nasal and buccal pressure sensors or thermistors is absent despite persistent respiratory effort which is assessed with detectors of abdominal and thoracic movement (Guilleminault & Dement 1978a).

- **Central:** in which there is complete cessation of airflow and diaphragmatic activity, which is indicated by a clear reduction in oesophageal pressure swings. This event should last 10 seconds or longer.
- **Mixed**: cessation of airflow and absence of any respiratory effort at the start of an episode, followed by resumption of the respiratory effort (Guilleminault & Dement 1978b). This type of breathing might be considered another variety of the obstructive pattern.

The average number of apnoeas and hypopnoeas per hour slept is generally referred to as the apnoea–hypopnoea index (AHI). An AHI of more than 5/hr when present along with daytime sleepiness or two other major symptoms (see below) has been used to define OSAHS (1999)

1.4 The Prevalence of OSAHS

Although the condition may occur in all age groups, including children, the focus has largely been on the middle-age population. Some studies have shown that OSAHS may increase with age (Bixler, Vgontaz, & Ten Have 1998) with a peak age of presentation around 50 years. The prevalence seems to diminish above the age of 60 years (Stradling & Davies 2004), especially if symptoms are not included in the definition. This may be because older patients may not complain of their symptoms or because it genuinely does not have such adverse consequences as in younger patients (Bixler, Vgontaz, & Ten Have 1998;Young, Palta, & Dempsey 1993). Nevertheless, Hoch and co-workers did not show any significant difference in daytime sleepiness between elderly healthy people (mean age 83+/- 3.1 years) and young subjects (mean age 25+/-3.1) (Hoch et al. 1992b). Furthermore, other studies suggest that the prevalence of obstructive sleep apnoea may increase with age

(Janssens et al. 2000;Ware, McBrayer, & Scott 2000) with an increase in AHI after a one-year follow-up (Hoch et al. 1992a).

Snoring has been reported in up to 60% of adults, yet only a small percentage of snorers develop OSAHS (Norton & Dunn 1985b;Ohayon et al. 1997). Based on the above-mentioned definition, the prevalence of OSAHS might be as high as 4% in men and 2% in women (Young, Palta, & Dempsey 1993). However, 24% of the US male population and 9% of women aged between 30 and 60 years have an AHI>5 without sleepiness, although the significance of this is unknown (Young, Palta, & Dempsey 1993).

1.4.1 Gender Differences

OSAHS is considered less common in women compared with men. Earlier studies have indicated that the prevalence of sleep apnoea in men is higher than in women in all age groups (Redline et al. 1994;Young & Finn 1998b). Nevertheless, Duran et al. in a study carried out in Spain did not show an obvious difference in prevalence between men and women. In this study, daytime hypersomnolence was found in 14% of men and 22% of women and it was not associated with age (Duran et al. 2001). It was also found that the habitual snoring in 46% of men and 25 % of women showed a significant tendency to increase with age. Breathing pauses during sleep were reported by 10% of men and only 2.5% of women and they increased with age. In addition, the difference in prevalence of OSAHS was not clear from polysomnography data based on variable AHI cut-off scores, which might indicate that the prevalence of asymptomatic OSAH is high in both sexes, although the variations in OSAHS might be due to differences in symptoms.

Any variation in prevalence between men and women might be attributed to the fact that the guidelines for the evaluation and diagnosis of OSAHS, established primarily for men, might not be valid for women. However, Young et al. found that the clinical indications for OSAHS evaluation are as appropriate for women as they are for men (Young et al. 1996). In a recent study from Pennsylvania and Madrid (Bixler, Vgontaz, & Lin 2001), the prevalence of OSAH was estimated based on a definition of hypopnoea requiring a 4% fall in SaO2 and AHI> 10. Symptoms were not required and thus OSAH rather than OSAHS was assessed. The total sample was 1,000 subjects, of whom 741 were men. Men had an OSAH prevalence of 3.9% and women 1.2%. However, in premenopausal women and postmenopausal women receiving hormone replacement therapy (HRT), the rate was 0.6% in contrast to 2.7% in postmenopausal women not receiving HRT. Furthermore, all premenopausal women and those receiving HRT who had an AHI > 15 had a BMI over 32. In contrast, 50% of postmenopausal women not receiving HRT had an AHI > 15, a similar frequency to that of men. These findings may perhaps be explained by the fact that female sex hormones are perhaps protective against OSAHS in non-obese women. Other studies had shown more severe OSAHS in postmenopausal women and reductions in indices of sleep disordered breathing from HRT (Dancey, Hanly, & Soong 2001). Conversely, testosterone may have a negative impact on OSAHS, perhaps by effects on the upper airway (Cistulli, Grunstein, & Sullivan 1994).

1.5 Physiology of Normal Sleep

Sleep is a reversible physiological and behavioural status. With the onset of sleep, the heart rate drops, cardiac output and blood pressure decrease, respiration slows,

and muscle tone is reduced. Sleep has two main divisions: rapid eye movement (REM) and non-rapid eye movement (NREM) sleep.

REM sleep periods occur about every 90 minutes throughout sleep and last around 20–30 minutes, occupying 20–25% of the night. It is characterized by intermittent horizontal eye movement, loss of postural muscle tone and fluctuation of autonomic functions with markedly variable breathing and surges in heart rate.

NREM sleep accounts for most of the sleep time in adults, about 75–80% of the night. It is categorized into four sleep stages identified using full polysomnography (see below). In stage 1, the EEG changes from alpha activity to low-voltage mixed frequency pattern (4–7 Hz). Stage 2 commences a few minutes after the onset of stage 1, with characteristic sleep spindles and K complexes. With the progress of sleep, high-voltage slow-wave activity may appear (2 Hz or less), which is the start of stages 3 and 4 or slow-wave sleep (SWS). Wakefulness may be referred to as stage 0.

There are usually 4–6 sleep cycles per night, with decreasing cycle length as the night progresses. The sleep cycle comprises wakefulness to stage1 all the way through to stage 4, then an episode of REM sleep. This pattern is repeated through the night with the periods of REM sleep gradually lengthening (in the rarely seen "classical" night of sleep), so that SWS predominates early, and REM sleep later in the night. Brief awakenings from sleep, known as arousals, may occur, which can be detected from EEG signals by abrupt shift in frequency to alpha or theta activities and last for about 10 seconds.

1.6 The Upper Airway in Health and OSAHS

1.6.1. The Normal Upper Airway

The upper airway consists of the nasal airway, the oral airway, the pharynx, the larynx and the trachea. The soft tissues and the skeletal components comprising these structures mainly determine the size of the upper airway. The pharynx is the main site of obstruction in OSAHS and extends from the back of the nose to the larynx. However, the nasal as well as the oral airway can also produce significant respiratory resistance. The patency of the pharynx is critically dependent on the action of the upper airway muscles: palatoglossus, palatopharyngeus, levator palatini, tensor palatini, stylopharyngeus, and salpingopharyngeus, which are active during respiration to prevent upper airway collapse. The muscles of the tongue also play a critical role, for the genioglossus, hypoglossus, and styloglossus, contribute to maintain the retroglossal airway (Miyamoto et al. 1997), although their effectiveness decreases with age (Mortimore et al. 1999). These upper airway muscles balance the intra-luminal pressure to keep the upper airway patent. During inspiration, the negative intra-luminal pressure tends to narrow the upper airway. This is opposed by the action of the upper airway dilating muscles, which tense with each inspiration, thus resisting collapse of the upper airway, the mechanism that is, at least in part, reflex-driven (Honer et al. 1991).

1.6.2 Upper Airway in Patients with OSAHS

OSAHS results from the narrowing of the upper airway during sleep and the site of the narrowing is usually in the pharynx. The pharyngeal airway of the OSA patient is smaller than that seen in controls, even in the wakeful state (Haponik, Smith, & Bohlman 1983;Martin, Marshall, & Douglas 1995;Schwab, Getter, & Hoffman

1993). In addition, some of the anatomical structures in the upper airway such as the tongue, uvula, pharyngeal fat pads and the lateral pharyngeal walls may be larger in the apnoea patient (Rivlin et al. 1984;Schwab, Getter, & Hoffman 1993). During sleep, and because of the negative pressure created during inspiration and the accompanying muscle hypotonia, the upper airway tends to narrow or collapse, causing apnoea or hypopnoea. Facial structure may also play a role in the pathogenesis of apnoea. Retroposition of the maxilla and mandible causes antero-posterior narrowing of the pharynx owing to posterior displacement of the soft plate and tongue (Jamieson, Guilleminault, & Partinen 1986;Riha et al. 2005). These facial abnormalities are particularly common in non-obese OSA patients and may run in families (Mathur & Douglas 1995b) .

1.7 Aetiology and Risk Factors

1.7.1 Genetic

Sleep apnoea is more common in family members of OSAHS sufferers than in the general population. Familial aggregation is generally explained by many risk factors involved in the pathophysiology of OSAHS being genetically determined. For example, twin studies have suggested that 70% of the variance in obesity within the general population can be attributed to genetic factors (Strohl et al. 1978). These factors which influence metabolic rate, thermogenesis, fat storage and distribution and eating behaviour, as well as facial structure (Mathur & Douglas 1995c;Riha et al. 2005) and endocrine and hypothalamic functions, are all thought to contribute to obesity and consequently to OSAHS (see below). Initial family studies addressed the issue of the genetic basis of OSAHS as it was reported in members of the same family in different generations (Mathur & Douglas 1995a;Redline et al. 1992).

Epidemiological studies (including twin studies) have evaluated the familial aggregation of symptoms of OSAHS. Kaprio et al. (Kaprio et al. 1988) found that the concordance for snoring was greater between monozygotic twins than dizygotic twins, suggesting a role of inheritance. Habitual snoring, excessive daytime sleepiness, gasping or apnoea was two or four times more frequent among first-degree relatives of patients with OSAHS than among control subjects, independent of familial similarities in body mass index (BMI), smoking, alcohol intake, age and gender (Redline et al. 1992). In the Copenhagen male study, the risk of snoring was increased approximately threefold when at least one first-degree relative was reported to be a snorer, and increased to fourfold when both parents were snorers (Jennum et al. 1995). Mathur et al. found that first-degree relatives of OSAHS patients have narrower upper airways with retroposed maxillae and mandibles and longer soft palates with wider uvulae (Mathur & Douglas 1995c), which suggest that OSAHS has a strong familial component.

The racial and ethnic variations in OSAHS are still not clear since relatively little is known about the disorder in non-Caucasian populations. However, data from the Cleveland Family Study and the San Diego study of the elderly demonstrated higher AHIs in African-Americans compared with Caucasians (Redline et al. 1995). This difference was not accounted for by differences in BMI, alcohol consumption or tobacco use, which were possible confounding factors. Community-based surveys in Singapore demonstrated that Chinese individuals had lower rates of snoring and sleep apnoea compared with Malays and Indians. After adjusting for obesity and age, the odds of sleep apnoea were reported to be approximately three and two times higher in Indians and Malays, respectively, compared with Chinese (Ng, Seow, &

Tan 1998). However, Ip et al. (Ip et al. 2001) found in a community-based study in Hong Kong that the prevalence of OSHA in Chinese people was 4.1% with AHI $\geq$ 5, increasing with body mass index (BMI) and age. In this study, she screened 1,542 men using a sleep questionnaire, although only 150 subjects had PSG. Among those who underwent PSG, 64 subjects (43%), 45 subjects (31%) and 37 subjects (25%) had AHIs $\geq$ 5, $\geq$ 10, and $\geq$ 15, respectively. Figure 1.1 demonstrates the findings of this study.

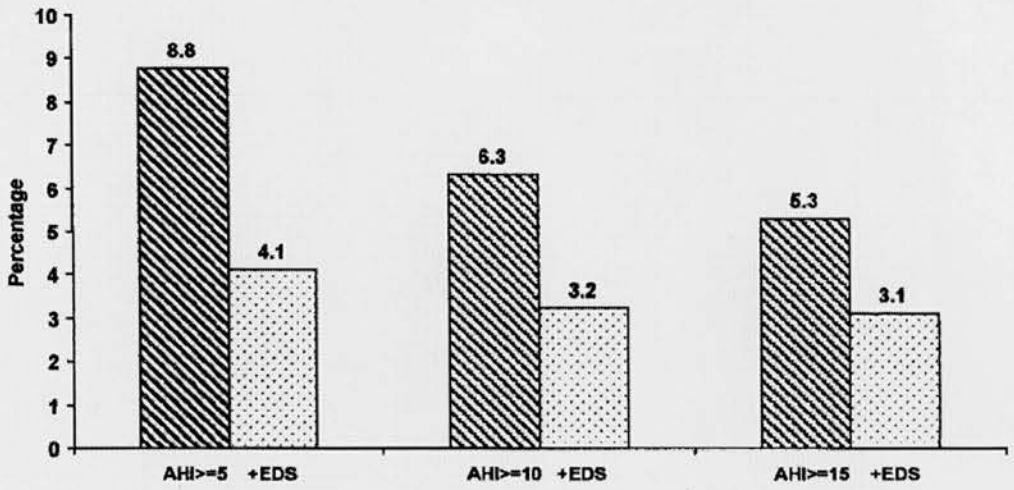


Figure 1.1 Prevalence rate of OSAHS in Chinese population (Ip et al. 2001)

1.7.2 Obesity

Approximately 50% of patients with OSAHS are obese, that is, their body mass index (BMI) is more than 30 kg/m2 (Douglas 2002a). In other studies, obesity appears to increase the risk of OSAHS by approximately 10—14-fold, mainly in middle-aged subjects (Guilleminault, Tilkian, & Dement 1976;Redline & Tishler 2000). The term syndrome Z has been introduced for the combination of hypertension, central obesity, insulin resistance, hyperlipidaemia and OSAHS (Wilcox et al. 1998). Obesity probably results in OSAHS from different mechanisms:

(1) increased fat deposition in the lateral nasopharyngeal and oropharyngeal areas, reducing its calibre (Horner et al. 1989;Suratt et al. 1983) and consequently altering upper airway function, rendering the pharynx more susceptible to collapse during sleep; (2) reduced chest wall compliance, causing hypoventilation owing to imbalance between mass loading and central drive (Strobel & Rosen 1996). Patients with sleep apnoea have difficulties in losing weight and often may gain weight because of daytime tiredness and consequent decrease in physical activity (Phillips, Kato, & Narkiewicz 2000). Furthermore, obese male patients with OSAHS have significantly higher plasma leptin (leptin regulates appetite and might play a role in the pathogenesis of obesity-related hypertension (Henriksen et al. 2000)) compared with matched obese controls who were free of sleep apnoea (Phillips, Kato, & Narkiewicz 2000). In another study, elevated leptin concentrations associated positively with skinfold thickness, hip/waist ratio, serum low-density lipoprotein cholesterol, and blood pressure in patients with sleep apnoea. In the same study, it was found that leptin concentrations decreased after 6 months of CPAP therapy (Ip, Lam, & Ho 2000).

1.7.3 Anatomical Factors

Retrognathia and micrognathia (congenital or acquired), including retroposition of the maxilla and/or mandible (Mathur & Douglas 1995c) and also deviation of the nasal septum all contribute to a reduction in upper airway size. Men and women with OSAHS have a lower-set hyoid bone than do those without OSAHS (Mathur & Douglas 1995c;Riha et al. 2005). In addition, enlarged tonsils and fat deposition in the upper airway (see above) may also narrow the upper airway. The supine position is associated with narrowing of the upper airway, perhaps owing to the tongue falling

backward, and consequently the severity of sleep apnoea increases in the supine posture (Douglas 2002b). Disorders such as acromegaly (Grunstein, Ho, & Sullivan 1991) and hypothyroidisim (Grunstein & Sullivan 1988) may reduce the upper airway size owing to excessive tissue thickness and thereby increase sleep apnoea.

1.8 Consequences of OSAHS

1.8.1 Hypertension and Cardiovascular Complications

There is now robust evidence that OSAHS is associated with hypertension. This is the main topic of this thesis and will be discussed in full in Chapter 2.

1.8.2 Pulmonary Haemodynamics Impairment

Haemodynamic consequences of OSAHS affecting the pulmonary circulation may lead to the development of pulmonary hypertension (PH). Niijima et al. (Niijima et al. 1999) evaluated 31 OSAHS patients with right cardiac catheterization and noted that pulmonary artery vasculature tone increased during REM sleep, independent of the degree of hypoxaemia, in patients with daytime pulmonary hypertension. The pathogenesis of PH in OSAHS could be attributed to several factors including hypoxia, hypercapnia and acidosis, neural reflexes and intrathoracic pressure. Hypoxia is probably the most important factor, since pulmonary artery pressure is increased by hypoxia (Cutaia & Rounds 1990). However, severe impairment of pulmonary haemodynamics occurs only in subjects with concomitant lung disease or morbid obesity (Kessler et al. 1996). OSAHS can also affect right ventricle (RV) morphology and function. Noda et al. (Noda et al. 1995) found RV hypertrophy and pulmonary hypertension in OSAHS subjects, in contrast to Sanner et al. (Sanner et al. 1997), who did not find that RV dysfunction was related to PH. Treatment of the apnoea could reverse the altered cardiopulmonary haemodynamics to some extent.

Fletcher found a substantial decrease in pulmonary artery pressure and significant improvement in right ventricular ejection fraction occurred in sleep apnoea patients, with and without concurrent lung disease, after tracheostomy. Pulmonary vascular resistance also decreased in these patients (Fletcher et al. 1987). There is no randomised controlled study showing that long-term CPAP treatment can reverse daytime pulmonary hypertension, although this is likely, but RV ejection fraction may increase after long-term CPAP treatment (Nahmias, Lao, & Karetzky 2004). More recently, it was found that severe OSAHS is independently associated with PH in direct relationship with disease severity and presence of diastolic dysfunction. Twelve weeks CPAP treatment reduced pulmonary systolic pressure (Arias et al. 2006).

1.8.3 Road Traffic Accidents

Patients with OSAHS are at a 1.3-7-fold greater risk of road traffic accidents (RTA) than the general population. Epidemiological studies have shown that patients with 15 apnoea/hypopnoea per hour or more are at risk of having around seven times more RTA than non-snoring normal subjects (Young et al. 1997). On direct questioning, many patients will admit to falling asleep at the wheel and some to having accidents or near accidents because of feeling sleepy while driving (Engleman, Hirst, & Douglas 1997a). Furthermore, there is evidence from vigilance tasks and driving simulators that driving performance is impaired in patients with OSAHS, similar to that of sleep deprivation (George, Boudreau, & Smiley 1996a;George, Boudreau, & Smiley 1996b;Hack et al. 2001). In a case-controlled study, Teran-Santos et al. (Teran-Santos, Jimenez-Gomez, & Cordero-Guevara 1999) in Spain studied the presence of OSAHS in all drivers aged 30 to 70 who received

emergency treatment after RTAs, and compared them with 152 age and sex-matched control subjects. They found that the subjects with AHI greater than or equal to 10 had unadjusted odds ratio of 6.3 for having RTAs and, quite surprisingly, for those with AHI greater than or equal to 5, the unadjusted odds ratio was 8.2. These findings remained significant after adjusting for age, alcohol consumption, medication use, visual refraction, BMI, driving history, and sleep schedule. Further evidence to support the link between OSAHS and motor vehicle accidents is that CPAP improves performance on vigilance tasks and the driving simulator and also reduced the road accidents rate by at least 40% in OSAHS patients (George 2001;Krieger et al. 1997).

1.8.4 Cerebrovascular Disorder

Many patients with stroke have sleep apnoea, which might either be a risk factor or a result of the cerebrovascular disease. Early epidemiological studies that examined the relationship between OSAHS and cerebrovascular disease, using self-reported snoring, show an association between snoring and stroke as an independent risk factor even when adjusted for age, gender, and obesity. The strength of this association is alleged to be similar to that for other risk factors of stroke, such as hypertension, smoking, atrial fibrillation and high cholesterol (Koskenvuo et al. 1987;Partinen & Palomaki 1985). However, stroke might cause sleep apnoea, since pharyngeal muscles can be affected in hemispheric stroke, and neurological dysphagia is seen in 30–40% of stroke patients (Barer 1989) or ventilatory control might be affected. A case-control study comparing 24 inpatients with recent stroke, confirmed clinically and radiologically, with 27 healthy subjects matched for age and gender (Dyke et al. 1996) with full polysomnography in all participants, reported

increased sleep apnoeas and hypopnoeas in 19% of the control group and 71% of the patients with stroke. The mean lowest arterial oxygen saturation level was 91% in the control group and 85% in the stroke group, and the mean AHI was 4 for the healthy individuals and 26 for the patients. Furthermore, obstructive apnoeas were more common than central apnoeas or Cheyne–Stokes respiration. This study indicates that OSAHS might be a consequence of stroke, although, in the absence of a study evaluating sleep apnoea preceding the stroke, it is difficult to be certain that OSAHS is a consequence of stroke. Nevertheless, a prospective study of 161 consecutive patients with first episodes of Transient Ischemic Attack (TIA) or stroke followed for 3 months (Parra et al. 2000) showed that there were no significant differences in the severity of sleep apnoea according to stroke subtype (TIA, ischemic or haemorrhagic stroke) or the location. This study also found that the frequency of OSAH did not significantly decline from the time immediately after the stroke to 3 months later, which might indicate that sleep apnoea preceded the cerebrovascular events. However, other studies have shown that sleep apnoea did improve with time and with CPAP treatment (Sandberg et al. 2001a;Wessendorf et al. 2001), indicating that OSAH is more likely to be in the main a consequence rather than a cause of stroke.

The presence of OSAHS after stroke is associated with a worse clinical outcome including early neurological worsening, delirium, depressed mood, impaired functional capacity, impaired cognition, and a longer period of hospitalization and rehabilitation (Good et al. 1996;Spriggs et al. 1992). The effect of CPAP in stroke prevention is not very clear, although its short-term effect after a stroke has been shown in a randomized study in which depressive symptoms were reduced in patients treated with nasal CPAP at 7 days and 28 days, compared with the untreated

controls. There was no significant improvement in delirium, daily living activities, or cognitive performance; while compliance for CPAP was about 50% only (Hui et al. 2002;Sandberg et al. 2001b;Wessendorf et al. 2001). Nevertheless, long-term compliance for CPAP in stroke patients is not certain, especially for those with more functional and cognitive disability (Sandberg et al. 2001a).

The pathophysiological mechanisms by which sleep apnoea could perhaps lead to the development of stroke are still not very clear. However, during sleep apnoea, hypoxaemia, hypercapnia, changes in intrathoracic pressure, and frequent sleep arousal, may elicit autonomic, haemodynamic, coagulopathic, and vascular injury processes that serve as plausible contributors to the development of stroke (Balfors & Franklin 1994). An observational cohort study by Yaggi et al,(Yaggi et al. 2005) found that obstructive sleep apnoea is an independent risk factor for stroke and sudden death with hazard ratio of 1.12-3.48; P=0.004. There is significant increased risk of stroke or death from any cause as a function of severity of OSAHS. The risk of stroke or sudden death in sleep apnoea patients is three times that of controls.

1.8.5 Cognitive Function Impairment

One of the clinical features in patients with OSAHS is impairment of the cognitive performance. Three-quarters of OSAHS patients reported difficulties at work and reduced work efficiency (Kales et al. 1985). The impairment might affect attention, concentration, vigilance, manual dexterity, visuomotor skills, memory, verbal fluency, and executive function (Engleman & Douglas 1993a). In a disease specific symptoms inventory, two-thirds of patients had memory disturbance and about 75% reported problems with concentration (Flemons & Reimer 1998). The assessment of cognitive performance in OSAHS can be conducted objectively, using different

physiological tests either manually or by computer. Several epidemiological (Kim et al. 1997;Redline et al. 1997) and clinical case-control studies (Bedard et al. 1991;Geenberg, Watson, & Deptula 1987) have been conducted to determine the state of cognitive function in those with sleep apnoea. These studies suggest that OSAHS of moderate severity could be associated with moderate to severe impairments of cognitive performance, although cognitive impairment may not be evident with mild sleep disordered breathing. Furthermore, studies have a shown marked improvement in cognition after CPAP treatment in moderate and severe OSAHS, but little change in cognition with CPAP in mild patients (Engleman et al. 1999f).

1.8.6 Quality of Life

Patients with OSAHS have a significantly impaired quality of life and social functioning, and a high prevalence of minor psychiatric morbidity (Grunstein et al. 1995b;Kales et al. 1985). These problems might be related to excessive sleepiness, low mood, motivation, and performance. One study suggested that more than two-thirds of severe OSAHS patients had problems with their work and family relationships (Kales et al. 1985). The Swedish epidemiological study (Grunstein et al. 1995a) also assessed the quality of life and showed that symptomatic OSAHS patients had a lower income, multiple divorce, and impaired work performance. Associated with the impaired quality of life may be minor psychiatric disorders such as anxiety and depressive disorders, which have been identified in approximately one-third to half of patients with OSAHS (Douglas 1998b;Engleman et al. 1993;Engleman et al. 1994b;Engleman et al. 1999c). Recent studies on quality of life have used generic scales to assess the functional limitations imposed by OSAHS.

These scales include the Nottingham Health Profile (NHP): the medical outcomes study Short Form-36 questionnaire (SF-36). There are also illness-specific scales such as the Calgary sleep apnoea quality of life index (SAQLI), and the functional outcomes of sleepiness questionnaire (FOSQ) (see Appendix). Research using generic and illness specific-illness scales suggests that the quality of life in OSAHS is reduced in aspects of both mental and physical functions. It has also been shown that the quality of life improves in OSAHS after CPAP treatment (Engleman et al. 1994a;Engleman et al. 1999b). In randomized controlled trials of patients with moderate to severe OSAHS, quality of life appears to improve with treatment as a function of initial impairment, with worst affected scores showing the greatest improvement (Engleman et al. 1999a;Jenkinson et al. 1999). In the randomized controlled trial of Jenkinson et al. very large improvements were seen in vitality score, with large effects on social function and moderate or small increments on other subscale scores (Jenkinson et al. 1999).

1.8.7 Polycythaemia

OSAHS patients with a co-existing condition causing daytime hypoxaemia may develop secondary polycythaemia. In a study of 624 patients (347 with an AHI > 10), Hoffstein et al. (Hoffstein et al. 1994) found that haematocrit levels and white cell counts increased minimally in patients with SaO2 < 85%, although there was no difference in haemoglobin levels. In addition, haematocrit decreased after one night of CPAP therapy, which might indicate a causal relationship between polycythaemia and OSAHS. However, there is no evidence that patients with OSAHS have increased erythropoietin levels (Pokala et al. 1995). The brief cyclical episodes of

hypoxaemia, typical of sleep apnoea, may not be sufficient stimulus for erythropoietin secretion (Goldman et al. 1991).

1.9 Diagnosis of Obstructive Apnoea/ Hypopnoea Syndrome

1.9.1 Clinical Symptoms

Daytime sleepiness is a cardinal feature of OSAS. This is often assessed by the diagnosis requiring patients to have a score of more than 11 on the Epworth Sleepiness Scale (see Appendix 1), which is the most widely used and best validated scale (Johns 1991b). Its advantages include ease of administration and low cost. It assesses the global level of sleepiness and is independent of short-term variations in sleepiness with the time of day and inter-day variations (Johns 2005). The ESS aims at measuring the general level of daytime sleepiness as a stable individual characteristic and has satisfactory test–retest reliability (Johns 1992b). The ESS is also able to discriminate between normal and pathological sleepiness (Johns 1994a). The severity of sleepiness can be divided into three levels:

1- Mild: unwanted sleepiness occurs during activities that need little attention such as reading, watching television or travelling as a passenger.

2- Moderate: the individual tends to have involuntary sleep during activities that require some attention such as during meetings or presentations.

3- Severe: involuntary sleep occurs during activities that require active attention such as during eating, walking, or driving, which may produce marked impairment in social or occupational function (The

Report of an American Academy of Sleep Medicine Task Force 1999).

Nocturnal features include snoring and witnessed apnoea (observed by the bed partner). In addition, patients may complain of choking, restlessness, nocturia and reflux (Senior et al. 2001). Other associated symptoms may include depression, daytime fatigue, decrease of libido, and impaired concentration (Douglas 2002a).

1.9.2 Clinical Examination

Measurement of height and weight and calculation of body mass index should be done for all patients with possible OSAHS who attend a sleep clinic. Many would measure neck circumference, which was found to be increased (averaging 43 _+ 4.5 cm) in patients with OSAHS (Hoffstein & Szalai 1993). The upper airway should also be examined to identify any structural abnormality that might cause upper airway narrowing such as retrognathia, macroglossia and tonsillar hypertrophy. The clinical assessment of the size of the upper airway was classified by Mallampati (Mallampati et al. 1985) into four grades (Mallampati score) based on the visualizing of the faucial pillars, soft plate and base of uvula:

Class 1: Entire tonsil clearly visible

Class 2: Upper half of tonsil fossa visible

Class 3: Soft and hard palate clearly visible

Class 4: only hard palate visible

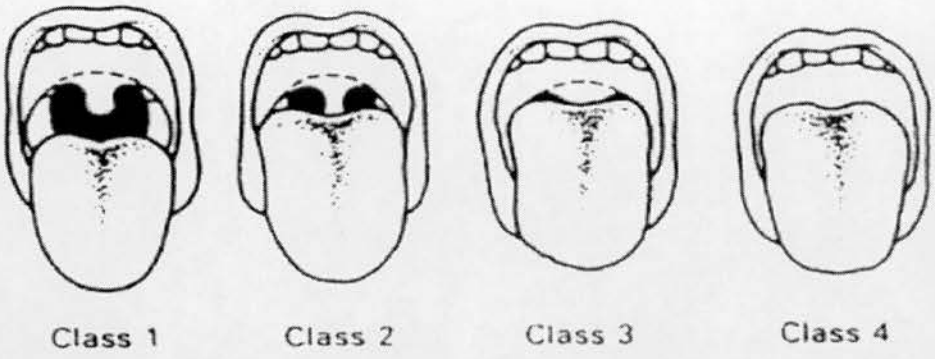


Figure 1.2 Classification of the Upper Airway after Mallampati (Mallampati 1983)

Obstructive sleep apnoea is associated with a higher Mallampati score (Hiremath et al. 1998). Furthermore, the uvula should be assessed for size, length and height; oedema and position. A low-lying palate and uvula are commonly seen in patients with OSAHS. The nose should also be inspected for any previous fractures, polyps or deviated septum as these may contribute to increased airway resistance and also for mask fitting in case of CPAP use (Douglas 2002b).

1.9.3 Laboratory Assessment

Full-night polysomnography (figure 1.3) is indicated for patients suspected of having sleep-related breathing disorders. Technically, polysomnography requires EEG, EOG, chin EMG, airflow, arterial oximetry, respiratory effort, and ECG. Anterior tibialis EMG is also useful to detect periodic leg movement, which might cause sleepiness.

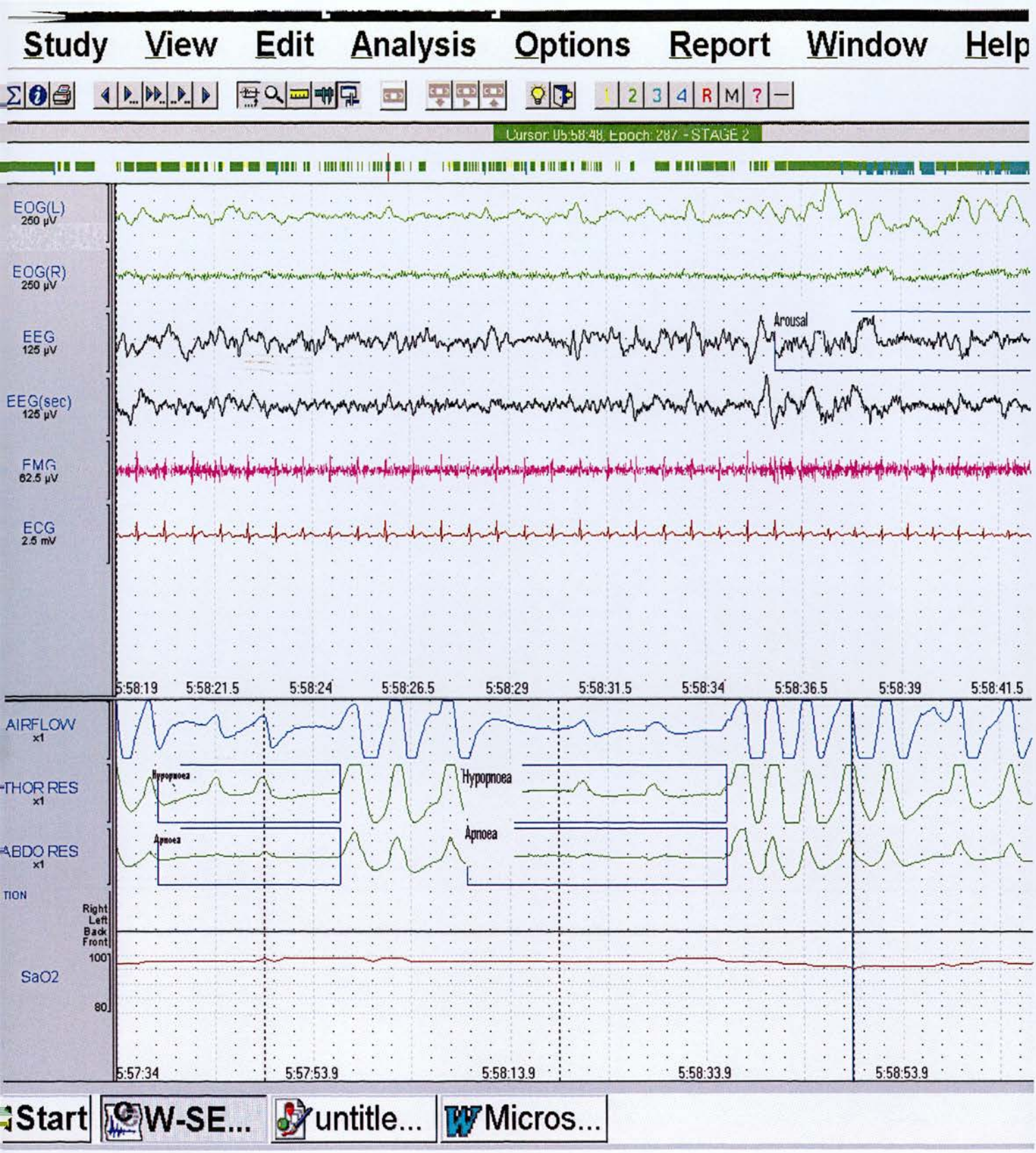


Figure 1.3 Example of full polysomnography

A limited sleep study, which is usually done at home, entails assessing some or all of respiratory movement, airflow, desaturation by means of oximetry, and heart rate without neurophysiological recording. There are several systems available and practice in this field is changing very rapidly (Douglas 2002a). Limited studies are usually done in the patient's home, to save sleep lab space and costs. Patients usually apply the sensors by themselves after instruction from sleep technicians. Some respiratory events occurring during wakefulness will be scored. However, limited sleeps studies are cost-effective and do not affect subsequent CPAP use (Whittle et al. 1997).

1.10 Treatment of OSAHS

1.10.1 General Measures

Overweight patients should be asked to lose weight, as weight reduction improves OSAHS (Smith et al. 1985). Unfortunately, sustained weight loss is rarely achieved by dieting alone (Pillar, Peled, & Lavie 1994). Weight reduction can be achieved by dietary advice or surgery such as jejeuno-ilial bypass surgery, gastric banding or gastroplasty. There are studies that have been conducted on the effects of surgical weight loss on OSAHS in which significant changes in AHI, nocturnal SaO2 and sleep integrity have been seen following surgery (Dhabuwala, Cannan, & Stubbs 2000;Pillar, Peled, & Lavie 1994;Strobel & Rosen 1996). However, these studies lack adequate long-term assessment as well as control studies. Patients should also be advised to avoid alcohol in the evening, and not to use sleeping tablets or sedatives, for all may decrease tone in upper airway dilating muscles.

1.10.1 Medical Therapy

1.10.1.1. Drugs

Different agents have been tried as a treatment option for OSAHS; unfortunately, however, there is no good evidence of benefit from any drug therapy.

Sex hormones. Progesterones are known to increase ventilatory drive and it has been suggested that medroxyprogesterone could allow better matching of diaphragmatic and upper airway tones (St John et al. 1986). Nevertheless, medroxyprogesterone had no positive effect on male patients with OSAHS in a well-designed double-blind, crossover trial (Cook, Benich, & Wooten 1989).

Acetazolamide inhibits carbonic anhydrase, producing a metabolic acidosis and an increase in respiratory drive. In a randomized, controlled trial, acetazolamide reduced the AHI by approximately half in 10 OSAHS patients, yet it did not improve arousal frequency or daytime somnolence (Whyte et al. 1988). Many patients developed adverse effects, causing withdrawal.

Serotonin agonists might be able, in theory, to increase upper airway dilating muscle tone. It has been shown that fluoxetine was more effective than protriptyline in reducing AHI, though with no reduction in daytime somnolence (Hanzel, Proia, & Hudgel 1991). However, there are no well-designed placebo-controlled studies to support its efficacy.

Physostigmine (PHYS) is an inhibitor of acetylcholinesterase activity. PHYS has a central effect with the induction of REM sleep by shortening the time of REM sleep onset and increasing the relative amount of REM sleep (Gillin et al. 1978). A randomized controlled study found that PHYS might reduce AHI in patients with moderate to severe OSAHS predominantly during REM sleep. However, the

reduction in AHI was only 21.3% and the average increase in Sao2 was 8.7% (Hedner et al. 2003).

Modafinil is a recently developed wakefulness-promoting, drug. Modafinil is chemically unrelated to amphetamines and it acts on specific areas in the anterior hypothalamus. This drug reduces persistent sleepiness in OSAHS patients complying with CPAP therapy and it is licensed in the UK for this purpose (Pack et al. 2001). However, its efficacy is limited and cost considerable. A randomized, double-blind, placebo-controlled study showed that Modafinil had no effect on sleepiness as measured by the ESS or the multiple sleep latency test compared with placebo in 30 OSAHS patients receiving effective CPAP therapy but still sleepy. However, a significant improvement in alertness was found using the maintenance of wakefulness test ($p < 0.02$) (Kingshott et al. 2001). CPAP use was significantly reduced by Modafinil compared with placebo ($p = 0.03$) (Kingshott et al. 2001). Nevertheless, Modafinil can be used as adjunct therapy for residual daytime sleepiness in sleep apnoea patients receiving CPAP therapy (Schwartz et al. 2003).

Other drugs have been tried without promising results or in inadequately controlled studies. These include theophylline, opioid antagonists, nicotine, antihypertensive agents, and clonidine; and currently they are not considered as a realistic treatment options for OSAHS.

1.10.1.2 Continuous Positive Airway Pressure (CPAP) therapy

CPAP was first described as a treatment for OSAHS in 1981 (Sullivan et al. 1981). It is delivered via a well-fitted mask applied to the nose or nose and mouth. The mask is attached to a machine that generates continuous pressurized air adjusted for the individual patient. CPAP acts as a pneumatic splint preventing the airway from

collapsing during sleep, thus reducing the apnoeas or hypopnoeas and subsequently improving sleep structure. In randomized placebo-controlled studies, CPAP has been shown to reduce sleepiness, improve quality of life (Douglas 1998c;Engleman et al. 1999d;Jenkinson et al. 1999), cognitive functions, mood disturbance (Douglas 1998a;Engleman et al. 1997a), driving performance (Krieger et al. 1997) and blood pressure (Faccenda et al. 2001a). These data apply to symptomatic patients with AHI more than 15. For symptomatic patients with AHI between 5 to 15 the benefit is not clear. Although CPAP may produce symptomatic improvement in this group there are only small improvements in cognitive function (Engleman et al. 1999e). In addition, mild sleep apnoea patients (AHI < 15 and ESS < 11) tend to use CPAP relatively poorly with only 50% continuing to use it after 3 years (McArdle et al. 1999b) compared with > 90% in patients with severe OSAHS (AHI > 30 and ESS > 15) . Nevertheless, CPAP use must be monitored objectively in all OSAHS patients, for patients tend to overestimate their use. The average CPAP run time in patient groups tends to be approximately three to four hours a night, although it may go up to five hours or more for regular CPAP clinic attendees (Engleman et al. 1997b;McArdle et al. 1999a). It is very important that patients receive adequate education, preferably along with their partners, about the rationale for CPAP and the need for long-term treatment. Optimal mask fitting with the most comfortable one is crucial prior to starting CPAP therapy, since the treatment is fairly obtrusive and these measures might improve CPAP usage and outcomes (Hoy et al. 1999).

The main side-effects of CPAP are nasal drying, stuffiness and rhinitis, which occur in about 60% of CPAP users (Engleman et al. 1996a). These symptoms are mainly due to mouth leaks causing high flows of cool air through the nose that can be

reduced by chin straps, changing to a full facemask or by using heated humidifiers (Massie et al. 1999;Richards et al. 1996). In addition, mask discomfort is a common complaint that can be managed with good fit and by avoiding excessive tightening of the straps. Abdominal bloating may also occur also, although it is rarely troublesome.

1.10.1.3 Mandibular Repositioning Splints (MRS)

Mandibular repositioning splints are designed to keep the lower jaw protruding during sleep, thus preventing the tongue falling back and narrowing the throat. Randomized placebo-controlled trials have shown that MRS use can decrease snoring (Stradling et al. 1998), apnoeas and hypopnoeas and improve symptoms in patients with mild OSAHS (Ferguson et al. 1996;Gotsopoulos et al. 2002;Mehta et al. 2001) and can also reduce blood pressure in OSAHS over 4 weeks of therapy (Gotsopoulos, Kelly, & Cistulli 2004). Nevertheless, it is less effective than CPAP in the same patients (Barnes et al. 2004;Engleman et al. 2002). The main side-effects of MRS include excessive salivation, mouth discomfort, crown displacement and temporo-mandibular joint discomfort (Pantin, Hillman, & Tenant 1999). MRS is less effective than 60% after 3 years. Reasons for stopping use include side-effects, social circumstances, dental treatment, as well as lack of perceived efficacy (Izci et al. 2005;McGown et al. 2001). At present, MRS devices are considered a good second line of treatment for patients with mild to moderate OSAHS who will not tolerate CPAP.

1.10.1.4 Pacing

There are attempts to pace the upper airway muscles as a form of treatment in OSAHS, such as pacing the tongue when upper airway obstruction occurs during sleep (Eisele et al. 1997). Because of the technical challenges of the pacing and lack

of a randomized controlled trial, this treatment cannot be considered an evidence-based option.

1.10.2 Surgery

1.10.2.1 Pharyngeal Surgery

The most common surgical operation used in OSAHS is uvulopalato-pharyngoplasty (U3P). The aim of the procedure is to widen the retropalatal airway by removing the uvula and part of the soft palate combined with tensing the lateral pharyngeal walls. This procedure can be done using different techniques, including scalpel cut, laser (LAUP) or diathermy. U3P may improve symptoms and breathing pattern (Li et al. 2003;Sher, Schechtman, & Piccirillo 1996), although none of these studies had adequate control groups and none was randomized. In addition, U3P is an invasive procedure and causes complications such as bleeding, stenosis, voice change, and velopharyngeal insufficiency that can make CPAP therapy difficult afterwards. Death has also occasionally been reported (Sher, Schechtman, & Piccirillo 1996). There are now attempts to improve the outcome of the procedure, such as using 3-dimensional computed tomography (3-D CT) measurements in evaluating the retropalatal space (Li et al. 2003) and also using modified (U3P) with extended uvulopalatal flap with more promising results (Li et al. 2003;Li et al. 2005). However, U3P results in decreased CPAP use by increasing mouth leak and reducing the maximal level of pressure that can be tolerated (Mortimore et al. 1996) which is a significant problem.

1.10.2.3 Other Upper Airway Surgery

1. Mandibular Maxillary Osteotomy (MMO)

MMO is a form of advancement of the mandible and maxilla by cutting both at an angle and rejoining them after sliding them forward (Hochban, Brandenburg, & Peter 1994). It is major surgery and performed by a maxillo-facial surgeon. It has been shown in a controlled trial that MMO is as effective as CPAP in normalizing the breathing pattern overnight and improving both symptoms and daytime vigilance (Conradt et al. 1998). However, there is as yet no clear indication about of when MMO should be performed, although, in general, this procedure should be considered for non-obese young patients and for patients with severe OSAHS who decline CPAP therapy.

2. Other surgical procedures

   There are other surgical procedures that can be employed in the management of OSAHS, such as debulking the base of the tongue, using laser mid-line glossectomy (Woodson & Fujita 1992), and advancing the insertion of the tongue by advancing the genial tubercle of the mandible (Riley, Powell, & Guilleminault 1994). These procedures are claimed to be effective in treating OSAHS although further data are needed.

1.11 Conclusion

OSAHS is a multi-factorial disorder with many implications, which may require more research work to understand its risk factors, pathogenesis, and association. It is very important to diagnose OSAHS, since treatment can improve the quality of life and prevent further complications. Although CPAP is the treatment of choice at present, other treatments can also be used, such as adjuvant drug therapy or, as an alternative, the surgical operations or using the MRS.

Chapter 2

Sleep Disorder Breathing and Hypertension

2.1 Introduction

When this work was started, there was an evidence that patients with sleep apnoea might be at increased risk of cardiovascular disease. Patients with sleep apnoea are often hypertensive (Davies et al. 2000;Pedulla et al. 1995) and up to one-third of hypertensive patients may have sleep apnoea (Berger, Somers, & Philips 2001). Several cross-sectional studies have shown that the prevalence of hypertension increases progressively with the severity of OSAHS (Lavie, Herer, & Hoffstein 2000;Nieto et al. 2000;Peppard et al. 2000). How hypertension is associated with OSAHS is not fully understood. However, repetitive episodes of airway occlusion during sleep, with consequent hypoxia, hypercapnia, dramatic changes in intrathoracic pressure and repeated arousals, may provoke a number of autonomic, haemodynamic, humoral and neuroendocrine responses. In this chapter, I will focus mainly on the autonomic responses and, in particular, the baroreflex changes that might occur in OSAHS patients. Other aspects of the possible vasculopathy in OSAHS will also be briefly reviewed.

2.2 Epidemiology

OSAHS and hypertension are associated. OSAHS and hypertension have common risk factors, such as obesity, alcohol intake, age, gender or exercise, which makes causation impossible to prove, using epidemiology alone.

2.2.1 In the General Population

Early studies of the association between hypertension and snoring came from Norton et al. and Koskenvuo et al., who found that snoring was a risk factor for hypertension

(Koskenvuo et al. 1987;Norton & Dunn 1985a). Sleep apnoea was an independent risk factor for the development of hypertension, similar to that of age and obesity (Carlson et al. 1994). A large epidemiological study showed that sleep apnoea significantly contributed to hypertension, independent of other risk factors (Lavie, Herer, & Hoffstein 2000). Each apnoeic event per hour of sleep added 1% to the risk of having hypertension. More evidence linking OSAHS with hypertension is provided by the Wisconsin Sleep Cohort study, which showed a dose-response association between OSAHS and *de novo* hypertension after 4 years of follow-up, independent of confounding factors, although the number of new hypertensive subjects was small (Peppard et al. 2000). In the original report, patients with an AHI > 25 had a fivefold risk of hypertension (Hla et al. 1994). The increase in risk of hypertension was greater in thinner patients who had abnormal breathing. After correction for confounding factors, those with an AHI > 15 had a 2.9-fold greater chance of developing hypertension in the following four years. A similar relationship between OSAHS and hypertension was found in the Sleep Heart Health Study (Nieto et al. 2000). In this study of 6,000 middle-aged and older adults, the prevalence of hypertension (defined as a resting BP $\geq$ 140/90 mmHg or the use of anti-hypertensive drugs) increased progressively with the severity of OSAH. After adjusting for the confounding factors, including obesity, the odds ratio in the group with severe OSAHS (AHI > 30) was 1.37 compared with those with lowest AHI (< 1.5). A cross-sectional study in a normal population by Bixler et al. also indicated an association between hypertension and sleep apnoea independent of other risk factors. The association was strongest in young subjects, and decreased with age (Bixler et al. 2000).

2.2.2 In the Hypertensive Population

Epidemiological studies of hypertensive patients have also suggested an association between sleep apnoea and hypertension. Hypertensive patients had a greater prevalence of sleep apnoea than normotensive subjects (Worsnop et al. 1998). Grote et al. found that OSAHS was a risk factor for poor blood pressure control in younger hypertensive patients (Grote, Hedner, & Peter 2000). A greater prevalence of obstructive sleep apnoea is found in adults with drug-resistant hypertension (BP > 140/90 who require a combination of three or more antihypertensive drugs) (Logan et al. 2001), supporting the idea of a possible role of OSAHS in the cause of hypertension.

2.2.3 In OSAHS

1-Animal models

In dogs, obstructive sleep apnoea leads to the development of sustained hypertension (Brooks et al. 1997). Obstructive sleep apnoea (OSA) was produced in four dogs using an occlusion valve attached to an endotracheal tube through which the dog could breathe. Obstruction of the airway by the valve was controlled by telemetry of ECG and EMG signals from the dog during a one- to three-month period. In the same dogs, sleep fragmentation was also induced. Arterial blood pressure was monitored for 12 hours every night. OSA caused a progressive increase in night-time mean arterial BP in each of the four dogs. There was no difference between the change in night-time BP caused by sleep fragmentation and that caused by OSA ($p = 0.4$). In contrast, the change in daytime BP caused by sleep fragmentation was significantly less than the change during OSA ($P = 0.001$). There were no changes in night-time or daytime heart rates during either OSA or sleep fragmentation. In

another dog study of chronic OSA by Parker et al. acute airway occlusion during sleep increased LV afterload and decreased fractional shortening. Chronic OSA caused a sustained decrease in LV systolic performance, caused either by systemic hypertension and/or transient increases in LV afterload during episodes of airway obstruction (Parker et al. 1999).

2-Human study

A case-control study found that patients with OSAHS had higher blood pressure than matched control subjects. Diastolic blood pressure in patients with OSAHS was significantly greater than controls during the daytime, night-time, and overall. OSAHS patients also had significantly greater night-time systolic BP ($p = 0.01$), although daytime and overall systolic blood pressure did not differ from control subjects (Davies et al. 2000). Direct evidence that OSAHS causes hypertension is provided by intervention studies, in which CPAP reduced BP (Faccenda et al. 2001c) (see below).

2.3 OSAHS and Blood Pressure

In healthy subjects, blood pressure normally decreases by 10% to 15% from its daytime value during sleep. This circadian drop in BP has been called dipping. However, some patients with OSAHS do not show nocturnal dipping of BP and are thus called nondippers (Pedulla et al. 1995). This may relate to apnoeas and hypopnoeas, which cause repeated nocturnal increases in BP, which consequently increase the mean sleeping BP (Pinto et al. 1993). The greatest pressure peaks occur after apnoea and may be 100 mmHg above the baseline value (Narkiewicz & Somers 1997;Somers et al. 1995). These acute nocturnal changes may lead to persistent daytime hypertension as a long-term consequence (Somers et al. 1995) with an

increased risk of target organ damage (Palatini et al. 1992). In a case-control study, sleep apnoea patients had significantly increased mean diastolic BP during both the daytime and night-time, and systolic BP was higher among OSAHS patients at night compared with controls. The nocturnal dip in BP was smaller in patients with OSAH than in matched control subjects (Davies et al. 2000).

Hypoxia may explain partly these variations in BP in sleep apnoea patients. In animals, repetitive episodic hypoxia causes diurnal elevation in BP in rats (Fletcher et al. 1992). Similarly rises in daytime blood pressure follow induced apnoeas in dogs and are probably related to hypoxaemia rather than arousal, because noise-induced arousal does not cause daytime hypertension in the same dogs (Brooks et al. 1997). Increased sympathetic activation, perhaps induced by hypoxaemia, may be a key factor in causing long-term BP changes (Lesske et al. 1997). However, other factors may be implicated in the development of hypertension in OSAHS patients, such as endothelial dysfunction, as discussed below.

2.4 Sympathetic Nervous System (SNS) in Hypertension

The sympathetic nervous system (SNS) has an integral role in the control of BP. Increased sympathetic activity can increase blood pressure through a variety of physiological and adaptive mechanisms. These include (1) increased vascular resistance caused by the release of noradrenalin in different vascular beds; (2) increased cardiac output; and (3) sodium and water retention by the activation of the renin-angiotensin system (Abboud 1982). The development of hypertension is partly due to abnormal renal excretory function, which is partly under the control of renal sympathetic nerve activity (RSNA). The renal effects of increased RSNA include increased renal tubular sodium reabsorption leading to renal sodium retention;

decreased renal blood flow and glomerular filtration rate with renal vasoconstriction and increased renal vascular resistance, and release of renin leading to the production of angiotensin II (Abboud 1982). Young human subjects with borderline hypertension have increased noradrenalin spillover from the kidney, which is an index of RSNA (Esler et al. 2001). Single-fibre RSNA is significantly greater in spontaneously hypertensive rats, than in normotensive Wistar-Kyoto rats (Thoren & Ricksten 1979). RSNA is affected by the arterial baroreflex, which may cause the immediate and sustained increase of arterial pressure. This is supported by studies of chronic unloading of the carotid baroreceptors in mongrel dogs. Ligation of the common carotid artery was used to cause baroreceptor unloading (Thrasher 2002). This procedure caused an immediate and sustained increase in main arterial pressure, an increase in heart rate and plasma renin activity, and a decrease in urinary sodium excretion, reflecting sustained increases in sympathetic nerve activity to the heart and kidneys (Thrasher 2003). Thus, progressive dysfunction of the arterial baroreflex during the development of hypertension may contribute to the sustained increase in activity of the sympathetic nervous system (including RSNA), and consequently arterial pressure.

2.5 Sympathetic Activity in OSAHS Patients

2.5.1 During Sleep

In normal sleep, heart rate, blood pressure, and sympathetic nerve traffic usually decrease (Somers et al. 1993). This reduction of sympathetic activity appears to increase progressively from stage 1 to stage 4 sleep (Narkiewicz & Somers 1997). However, during REM sleep, sympathetic activity increases, to as much as double that of wakefulness (Somers et al. 1993). Blood pressure and heart rate during REM

are variable, but average about the same as during wakefulness (Narkiewicz & Somers 1997).

In contrast, sympathetic activity is increased during "sleep" in OSAHS patients and the sympathetic and the haemodynamic state during "sleep" is determined primarily by the duration and severity of apnoea rather than by the sleep stage itself (Somers et al. 1995). Repetitive episodes of obstructive apnoea, hypoxia and hypercapnia probably act through chemoreceptor reflexes and other mechanisms to increase sympathetic drive (Morgan et al. 1995a). Resumption of breathing results in increased venous return and increased cardiac output. This increased cardiac output is delivered into a severely constricted peripheral vasculature, with surges in BP (see above).

2.5.1.1 Effects of Arousal from Sleep on BP

Normal spontaneous arousals from sleep are associated with transient increases in blood pressure, heart rate and ventilation (Horner 1996). These increases are caused by changes in sympathetic activity caused by the arousal. In a study in dogs (Schneider et al. 2000), ventricular stroke volume (SV) remained constant when apnoea ended, if there was no arousal. However, with arousal from apnoeas, heart rate and cardiac output increased, although SV decreased. Arousal increased the systemic but not the pulmonary arterial pressure in response to obstructive apnoea. The increase in systemic blood pressure was more marked during NREM sleep than in REM sleep (Schneider et al. 2000). In a large population-based study, sleep fragmentation index (SFI; calculated as the total number of awakenings/shifts to stage 1 divided by the total sleep time per hour) was significantly associated with systolic but not diastolic blood pressure during wakefulness in individuals with AHI

< 1. However, sleep fragmentation and blood pressure were not associated in those with AHI > 1 after controlling for the influence of the AHI. The author concluded that sleep fragmentation was independently associated with a greater wakefulness systolic blood pressure during wakefulness (Morrell et al. 2000). Noda et al. (Noda, Yasuma, & Yokota 2000) found that end-apnoeic arousal and hypoxic asphyxia and the subsequent sleep fragmentation might contribute to nocturnal and diurnal elevation of BP. The rise in blood pressure with arousal might be caused by increase in sympathetic activity. Sympathetic outflow remained elevated for a substantial period even after a hypoxic stimulus was removed (Morgan et al. 1995b). Nevertheless, it is unwise to conclude that sleep arousal is the sole contributor to sustained hypertension in awake sleep apnoea patients. However, in patients with higher AHI, sleep disruption may modulate the BP along with other effects such as hypoxaemia and changes in intrathoracic pressure, which may overcome the effects of arousal (Morrell et al. 2000).

2.5.2 During Wakefulness

Greater sympathetic activity in OSAHS patients may be present even during daytime wakefulness, when subjects are breathing normally and both arterial oxygen and carbon dioxide levels are normal (Hedner et al. 1988;Somers et al. 1995). Circulating catecholamines (Marrone et al. 1993) and muscle sympathetic nerve activity (Narkiewicz et al. 1998a) were greater in patients with OSAHS compared with normal subjects, probably because of baroreflex dysfunction, chemoreflex excitation, and endothelial dysfunction (see below). Greater sympathetic drive in these patients may contribute, to a certain extent, to chronic elevated resting BP. The mechanism underlying the sustained increase in sympathetic drive is not clear. Morgan et al.

suggested that combined hypoxia and hypercapnia evoke long-lasting sympathetic activation (Morgan et al. 1995a). This may explain in part the increased daytime sympathetic drive in OSAHS patients. However, repeated BP increases, acting via the baroreceptors, may reset the baroreflex, permitting a higher level of sympathetic activity and BP even during wakefulness. To understand the role of the chemoreflexes and baroreflexes in BP control in OSAHS patients, I will discuss these two aspects in detail below.

2.5.3 Chemoreflexes

The chemoreflexes are important and powerful modulators of sympathetic activation. Hypoxia, which acts primarily on the peripheral chemoreceptors located in the carotid bodies (Daly, Angell-James, & Elsner 1979), and hypercapnia, acting on the central chemoreceptors located in the brain stem, trigger reflex increases in minute ventilation as well as sympathetic activity. Patients with OSAHS have an enhanced vascular response to hypoxia (Narkiewicz et al. 1998c). In a double-blind, randomized, vehicle-controlled trial, it was found that MSNA and mean arterial pressure were significantly reduced in OSAHS patients compared with control subjects during chemoreflex deactivation by 100% oxygen (Narkiewicz et al. 1998c). However, the enhancement of peripheral chemoreflexes are selective with autonomic, haemodynamic and ventilatory responses in normotensive OSAHS (Narkiewicz et al. 1999c). Furthermore, this enhancement of the reflex response to hypoxia is not explained by obesity, since obese subjects who are otherwise healthy with no OSAHS have chemoreflex responses similar to those seen in control subjects (Narkiewicz et al. 1999a;Narkiewicz et al. 1999c). Nevertheless, obese patients had a greater response to hypercapnia (Narkiewicz et al. 1999a). Both hypoxia and

hypercapnia have local vascular effects, causing vasodilation, which lowers the blood pressure initially, which in turn increases sympathetic activity (3) and catecholamine release (Shepard, Jr. 1990). During apnoea, sympathetic activity rises gradually, reaching its peak at the end of the apnoea, when oxygen desaturation and carbon dioxide retention are most marked (Somers et al. 1995). On release of the airway obstruction and resumption of breathing, increased cardiac output, together with the constricted peripheral vasculature, result in a marked increase in blood pressure (Somers et al. 1993). There is also a carry-over effect to the tonic activation of the peripheral chemoreceptors, even during normoxia, which may partly explain the increased sympathetic activity during the daytime (see above).However a double-blind study suggested that hyperoxia can suppress peripheral chemoreceptors in OSAHS patients, shown by a decrease in blood pressure and slowing of heart rate (Narkiewicz et al. 1998c).

2.6 Baroreflex Physiology

2.6.1 Arterial Baroreflex

The baroreceptor heart rate reflex, or baroreflex, is the fastest blood pressure buffering mechanism (Guyton & Hall 2000). The arterial baroreflex seeks to regulate the absolute blood pressure and to maintain the circulation to the brain and other organs. Baroreceptors sense systemic blood pressure indirectly, by the stretch of receptors in the walls of the carotid arteries and the aorta. A rise in blood pressure elicits reflex parasympathetic activation and sympathetic inhibition with subsequent decrease in heart rate (HR), cardiac contractility, vascular resistance, and venous return. Conversely, a decrease in arterial pressure reduces baroreceptor afferent discharge and triggers sympathetic stimulation with an increase in HR and peripheral

vascular resistance, leading to an elevation of arterial pressure. In the following section, I shall discuss briefly the basis of baroreflex regulation of arterial blood pressure.

Mayer waves

Mayer waves are oscillations of arterial pressure occurring spontaneously in conscious subjects at a frequency lower than respiration (0.1 Hz). These spontaneous oscillations can be shown in experimental animals and humans, using spectral analysis techniques. In one experiment on adult cats (Preiss & Polosa 1974), an episode of Mayer waves was considered an oscillation of systemic arterial pressure lasting for five minutes without attenuation. This oscillation is independent of and slower than the rate of animal's respiratory centre activity. In addition, the arterial pressure oscillation is associated with the simultaneous oscillation of sympathetic neural activity. Preiss and Polosa found that Mayer waves were the result of the activity of a central oscillator (Preiss & Polosa 1974). The Mayer waves persisted even after systemic arterial pressure stabilization or decrease. However, some authors consider that the baroreflex loop could be important in causing Mayer waves. Arterial pressure oscillation is attenuated after surgical denervation of aortic and carotid baroreceptors (Di Rienzo et al. 1991;Mancia et al. 1999). If the baroreflex loop was interrupted in humans with the use of the alpha-adrenoreceptor antagonist; phentolamine, Mayer waves and the oscillation of the sympathetic nervous system were strongly depressed (van De et al. 2001). The amplitude of Mayer waves may vary considerably within a particular individual over time (Janssen et al. 1997). There appears to be a positive relationship between the amplitude of Mayer waves and the strength of the corresponding sympathetic nervous system oscillations

(Furlan et al. 2000), and these relationships have limited within-subject reproducibility in the long term (Taylor et al. 1998), which may affect the reproducibility of baroreflex measurements (see Chapter 8 on Reproducibility).

2.6.2 Cardiopulmonary Baroreflex

This baroreflex arc includes stretch fibres in the heart and lungs, which act as volume sensors relaying information about the central blood volume to the brain stem, where signals are integrated with those from arterial baroreflex to modulate sympathetic outflow.

2.6.3 Physiological Influences on Baroreflex Function

1- Respiratory influence on baroreflex function

Respiration modulates cardiac autonomic regulation. Inspiration decreases and expiration increases the cardiac vagal traffic caused by baroreflex activation. Hyperventilation impairs the baroreflex influence of the heart rate (HR) and sympathetic nerve traffic. Respiratory activity changes the membrane potentials of preganglionic vagal and sympathetic motor neurons and continually modulates their responsiveness to stimulatory inputs. This process is called respiratory gating and is seen in the rhythmic fluctuation of R-R intervals (Ekberg 2003). This feature is discussed in depth in Chapter 8 on Reproducibility.

2-Neurohumeral influences

Various hormones influence baroreflex function. Angiotensin II resets the baroreflex function curve to a higher pressure independent of its effect on arterial pressure. In contrast, vasopressin resets the baroreflex curve to a lower pressure. These actions are mediated to some extent by the effect of these two circulating peptides on the area postrema, a circumventricular region that lacks blood brain barrier and contains

neurons that project to the vasomotor control centres (Guyton et al. 1972;Huang & Leenen 1999).

3- **Ageing**

Ageing is associated with significant cardiovascular modifications, and arterial baroreflex modulation of HR and sympathetic activity may decrease with advancing age (Gribbin et al. 1971). Vascular compliance, which might decrease with age, may decrease the baroreflex sensitivity (Gerritsen et al. 2000).

4-Physical Deconditioning

Orthostatic intolerance is the major cardiovascular effect of deconditioning after a prolonged bed rest or microgravity in space flight, which might be caused by autonomic dysfunction. Other factors such as hypovolaemia or vascular dysfunction may also contribute to orthostatic intolerance (Moffitt et al. 1998). After head-down bed rest for 14 days, baseline arterial baroreflex regulation of HR is reduced (Kamiya et al. 2000b;Kamiya et al. 2000a).

5-Gender

There is a small difference between men and women in BRS. Huikuri et al. found that middle-aged men have higher BRS values (10.5 ± 4.6 ms/mmHg) than middle-aged women (8.0 ± 4.6 ms/mmHg) (Huikuri et al. 1996) and women receiving hormonal replacement therapy (HRT) had higher BRS values that women without HRT. However, younger women showed higher BRS during preovulation phase of their menstrual cycle, compared with early follicular and midluteal phases but similar to those of age- matched men (Tanaka et al. 2003), which could be attributed to an effect of oestrogens (Liu, Kuo, & Yang 2003).

2.6.4 Baroreflex and Hypertension

There is evidence that the cardiac baroreflex is impaired if blood pressure is increased, in both humans (Floras et al. 1988;Siche et al. 1995) and animals (Lantelme, Lo, & Sassard 1994). Floras et al. found that the arterial baroreflex could buffer acute changes in blood pressure in subjects with WHO stage 1 hypertension. However, this ability is weakened if the BRS is reduced. With the development of clinically evident cardiac adaptation to hypertension (WHO stage 2), the contribution of the arterial baroreflex to the regulation of blood pressure is no longer detectable and the influence of cardiac and somatic afferents to reflex circulatory adjustment to activity may predominate (Floras et al. 1988). Furthermore, Lantelme et al. found that hypertensive rats have impaired cardiac baroreflex responses, characterized by a range-independent decreased gain, which is not caused by cardiac hypertrophy (Lantelme, Lo, & Sassard 1994). The impaired baroreflex may even precede the development of hypertension. Baroreflex inhibition of muscle sympathetic nerve activity is reduced in adolescents with a family history of hypertension, even when they were normotensive, which may lead to the development of hypertension by increasing sympathetic vasomotor tone (Yamada et al. 1988). This could also be a factor in hypertension in OSAHS patients, although there is no evidence for this so far.

2.6.4 Baroreflex in Sleep Apnoea

Patients with OSAHS have baroreflex dysfunction. Narkiewicz et al, used phenylephrine to activate baroreceptors and nitroprusside to deactivate them. Normotensive patients with OSAHS had an impaired response to baroreceptor deactivation but not to baroreceptor activation. They suggested that the reduced

baroreflex sympathetic modulation in patients with sleep apnoea was not accompanied by any impairment of baroreflex control of heart rate (Narkiewicz et al. 1998b). In addition OSAH patients have impaired baroreflex responses to a hypotensive stimulus (Carlson et al. 1996). Furthermore, it was found by using sequence method analysis (Bonsignore et al. 2002;Tkacova et al. 2000), that baroreflexes are impaired in OSAHS patients compared with healthy controls.

2.7 Effect of treating Sleep Apnoea on Sympathetic activity and BP

As discussed in Chapter 1, treating of sleep apnoea improves symptoms. In addition to symptoms, CPAP treatment may also reduce sympathetic activity. Nasal CPAP was found to reduce catecholamines (Jennum et al. 1989). Somers et al. found that CPAP treatment caused an acute and marked reduction in nocturnal sympathetic nerve traffic (Somers et al. 1995). However, CPAP does not reduce daytime blood pressure acutely, although it significantly reduces the large oscillations in blood pressure seen overnight in patients with untreated sleep apnoea (Ali et al. 1992). Nevertheless, a small fall in night-time systolic BP was seen in OSAH patients after 2 weeks of treatment (Davies et al. 1994), with some improvement in daytime mean arterial blood pressure in non-dippers after 3 weeks of CPAP treatment compared with placebo (Engleman et al. 1996b). However, when effective CPAP treatment was given for a longer period (8 weeks) in a before-and-after non-placebo controlled design, there was a significant fall in both systolic and diastolic BP, independent of changes in body weight (Wilcox et al. 1993). Thus, long-term treatment with CPAP may be needed to attenuate sympathetic activation and consequently reduce BP. This idea is supported by the findings that CPAP treatment reduced the muscle sympathetic nerve activity (MSNA) in otherwise healthy OSAHS patients, although

the reduction was evident only after one and a half years of treatment (Narkiewicz et al. 1999b). Furthermore, in the only randomized placebo-controlled crossover study published before the work on this thesis started, Faccenda et al. found that CPAP therapy reduced 24-hour diastolic blood pressure in comparison with the placebo, although the overall reduction was small, averaging 1.5 mmHg over the 24 hours. The decrease was greater during the early morning period, that is, 2:00 a.m. As predicted a priori, the decrease was greater in those with more nocturnal hypoxaemia (> 20% desaturations/hour) (Faccenda et al. 2001b).

2.8 Heart Rate Variability (HRV)

The beat of the healthy heart is not regular. It varies as a result of many factors, including exercise and physical and mental stress. In addition, the intervals between normal sinus beats vary periodically because of respiration, blood pressure regulation (see above), thermoregulation, actions of the renin-angiotensin system, and circadian rhythms (Stein et al. 1994). HRV can be measured by a non-invasive method of power spectral density (PSD) analysis. PSD describes how the energy (or variance) of a signal or a time series is distributed with frequency. There are two ways to measure the HRV, the time domain and the frequency domain.

Time domain indices use analysis of interbeat intervals or a comparison of the lengths of adjacent cycles, in relation to time. Such indices include the standard deviation of all normal R-R intervals and the standard deviation of the mean of the 5-minute intervals, averaged over a 24-hour period. A geometric approach to quantifying inter-beat interval-based HRV entails measuring the baseline width (in msec) of the main triangle superimposed on the histogram of all inter-beat intervals (Task Force 1996)

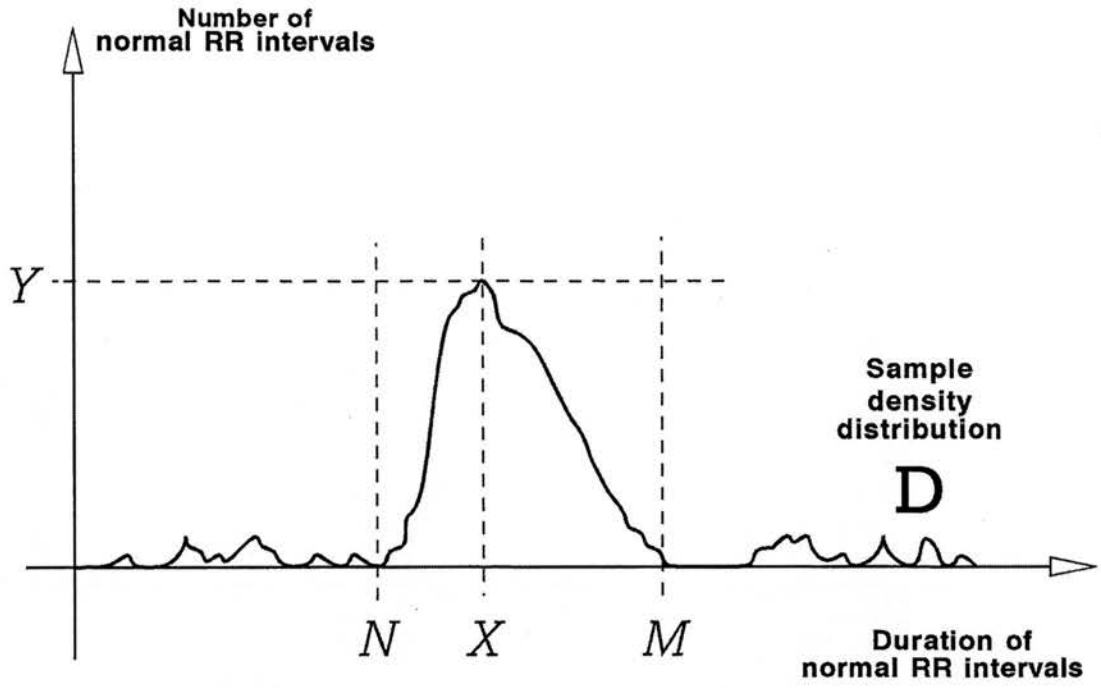


Figure 2.1 Time domain of the heart rate variability. Normal R-R intervals are all intervals between adjacent QRS complexes resulting from sinus node depolarization.
Adapted from "Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology", *Circulation* (1996),93:1043–1065).

This method has the advantage of being less dependent on the accurate classification of individual beats. The second class of time domain variables is based on comparisons of lengths of the adjacent cycles, including the proportion of the adjacent cycles that are > 50 msec apart, measured as a percentage, and the root mean square successive differences. These variables are virtually independent of long-term trends and predominantly reflect vagal tone.

The frequency domain gives information about the amount of overall variance in heart rate resulting from periodic oscillations of heart rate at various frequencies.

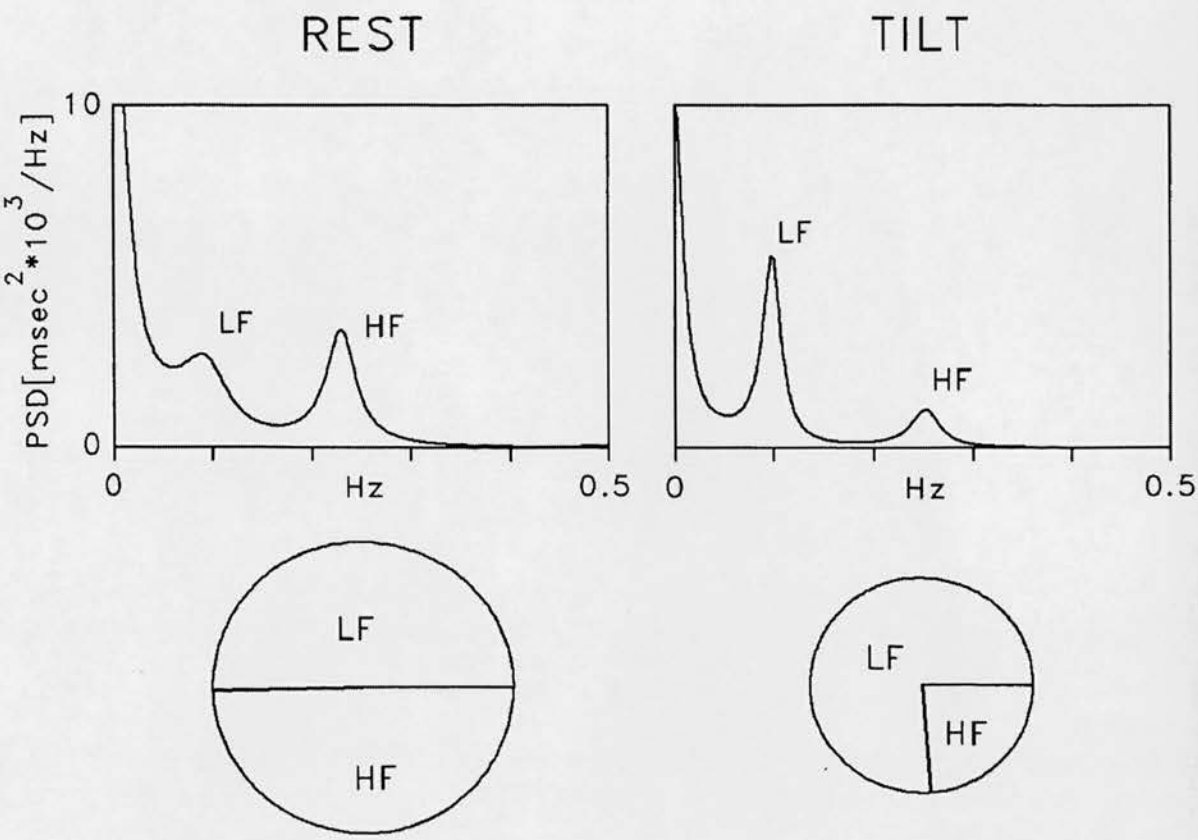


Figure 2.2 Frequency domain of the heart rate variability in two different positions, adapted from "Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology", *Circulation* (1996),93:1043–1065).

The variance of the heart rate is referred to as 'power' in a portion of the total spectrum and is measured in milliseconds squared. The power spectrum has been classified into three bands: high frequency power (HF), which is parasympathetically mediated and represents mainly the respiratory variation; low frequency power (LF), which is modulated by both the sympathetic and parasympathetic nervous systems and strongly affected by the oscillatory rhythm of the baroreceptors (Bigger et al.

1992;Yu & Lumbers 2000); and very low frequency power (VLF), which may represent the influence of the thermoregulation, peripheral vasomotor, or renin-angiotensin systems. The total power (TP) is the sum of all these three frequencies (see above).

Time and frequency domain measures of HRV are closely related, since for every frequency domain measure, there is a time domain measure that is strongly correlated with it. Indices of HRV have been shown to be stable, at least over a 30- to 65-day interval, and there is no placebo effect on HRV (Kleiger et al. 1991).Thus, the measurement of HRV is an excellent tool for studying autonomic input to the heart (1996a)

2.9 OSAHS and Endothelial Function

2.9.1 Introduction

The endothelium is the cell layer lining the blood vessels. It is one cell thick and senses changes in haemodynamic states (Raitakari & Celermajer 2000). The endothelium responds to physical and chemical stimuli by synthesis or release of substances such as nitric oxide (NO), prostacyclin, endothelins, endothelial cell growth factors, interleukins, adhesion molecules, and fibrinolytic factors (Jaffe 1987). It controls thrombosis and thrombolysis, platelet and leukocyte interactions with the vessel wall, and regulation of vascular tone and growth that play a very important role in cardiovascular control. The endothelium has anticoagulant, antiplatelet, and fibrinolytic properties. Endothelial cells are the major sites for anticoagulant reactions involving thrombin. Platelet adhesion to endothelial cells is markedly inhibited by the endothelium-derived arachidonic acid metabolite, prostacyclin. The same stimuli that activate platelets, such as thrombin, also act to

release prostacyclin and tissue-type plasminogen activator (TPA) from the endothelium, which allows the normal endothelium to limit the extent of platelet plug formation. The endothelium also controls underlying smooth muscle tone in response to certain substances. These substances include endothelium-derived relaxing factor (EDRF), mediated by NO, acetylcholine, endothelin, and substance P (Jaffe 1987;Vanhoutte 1989).

2.9.2 Nitric Oxide (NO)

Nitric oxide is a gas with a half-life of several seconds in biological tissues. It is synthesized from L-arginine by an enzyme, NO synthase (NOS) (Palmer, Ferrige, & Moncada 1987). Three distinct isoforms of NOS have been identified. Two of these are constitutively expressed: endothelial NOS (eNOS), and neuronal (nNOS). The third iso-form is inducible NOS (iNOS) and is regulated by cytokine stimulation (Nathan & Xie 1994). NO, a potent inorganic vasodilator, acts of blood vessels to maintain a low vascular tone at rest in both the systemic and pulmonary circulation (Faller 1999). It inhibits platelet aggregation and adhesion as well as smooth muscle fibroblast mitogenesis (Garg & Hassid 1989). In addition, NO plays an important role in vascular growth, leukocyte adhesion, immunological regulation, metabolism of circulating amines, lipoprotein metabolism, and integration and transduction of blood-borne signals (Riatakari & Celermajer 2000).

NO transmits its message by activation of soluble guanylyl cyclase, which then produces cyclic GMP (cGMP). cGMP appears to be the second messenger responsible for mediating vasorelaxation and antiplatelet functions (Nathan & Xie 1994). NO can also act directly on calcium-dependent potassium channels, causing relaxation of smooth muscle. The vascular effect of NO is confined not only to the

smooth muscle cell, but may also include promotion of endothelial cell proliferation, protection of endothelial cells from apoptosis, and inhibition of adherence of inflammatory cells (Furchgott & Zawadzki 1980). NO has been implicated in the pathogenesis of many cardiovascular diseases, including atherosclerosis, hypertension, intimal hyperplasia, and aneurysmal disease (Rubanyi 1993). This section will examine mainly atherosclerosis and hypertension as the main focus of this thesis.

2.9.2.1 Atherosclerosis and NO

Disturbed endothelial function may be important in cardiovascular disease. Atherosclerosis results from excessive inflammatory and fibroproliferative responses to vascular insults. The earliest change in the vessel wall is the formation of the fatty streak and monocyte adhesion (Gerrity 1981). Patients with developing atherosclerosis have reduced NO bioavailability in both the peripheral and coronary vasculature (Rubanyi 1993). The importance of NO in atherogenesis was suggested in mice deficient in apolipoprotein E (apo E), in which atherosclerotic lesions developed spontaneously when eNOS was deleted (Barbato & Tzeng 2004;Bocksch et al. 2001;Rubanyi 1993). Specific factors can alter the production of NO and therefore influence the progression of atherosclerosis. BH4 is a necessary cofactor for the proper functioning of all NO enzymes. When it is in short supply within the cell, NOS uncouples and behaves as an NADPH oxidase, leading to the production of superoxide rather than NO (Kitamoto et al. 2000). Hypercholesterolaemia and hyperlipidaemia mediate endothelial dysfunction, in part by consuming NO and forming reactive oxygen species and consequently contribute to the atherosclerotic plaque formation (Ross 2004).

2.9.2.2 The Role of Hypoxia in NO Production

Hypoxia has been associated with both the upregulation (Justice, Tanner, & Myers 2000;Resta et al. 1999) and downregulation (Quinlan et al. 2000) of eNOS expression. It seems that NO regulation varies among vascular beds as well as between species (Tai, Robb, & Marsden 2003). *In vivo,* hypoxia induces upregulation of eNOS gene expression and consequently NO production (Hoffman, Gloe, & Pohl 2001;Thompson et al. 2000). In a study assessing the cell regulation of NO production during hypoxia in porcine coronary microvessels and epicardial arteries, hypoxia caused a time-dependent increase in eNOS protein when compared with cells grown under conditions of normoxia. The NO production was markedly increased after 30 minutes of exposure to low oxygen (Justice, Tanner, & Myers 2000). In contrast, other studies have shown that eNOS is downregulated by prolonged hypoxia in human or bovine cultured endothelial cells (Phelan & Faller 1996). Faller et al. found that low oxygen tension (PO2 = 20 - 40 mmHg) results in a profound decrease in the transcript of eNOS, a corresponding fall in eNOS protein levels, and co-ordinated impairment of production of NO in response to stimulators of eNOS (Faller 1999). In another study, using rat pulmonary artery, both acute and chronic hypoxia selectively attenuated pulmonary endothelial NO production (Shaul, Wells, & Horning 1993). It is perhaps not surprising that endothelial cells from various vascular beds should respond to hypoxia differently, depending on the specific *in vivo* and *in vitro* milieus, as well as the chronicity and severity of the hypoxic exposure (Tai, Robb, & Marsden 2003).

2.9.2.3 NO and Hypertension (Endothelial dysfunction)

Endothelial dysfunction is a reversible change in endothelial cells, which may result from impairment in NO availability. Thus, endothelial dysfunction is distinguished from endothelial damage, which is represented by the anatomical disruption of the endothelium. Inhibition of NO production produces hypertension in animals and humans. In animal models, eNOS knockout mice developed mild to moderate hypertension, with blood pressure levels 20 mmHg higher than in normal controls (Huang et al. 1995). When normotensive mice were engineered to overexpress eNOS, their mean BP levels were 18 mmHg lower than normal (Ohashi, Kawashima, & Hirata 1998). In humans, blocking of eNOS by methylated arginine caused a marked and dose-dependent elevation of BP, suggesting that production of NO is one of the most important factors in BP regulation. Furthermore, endothelial dysfunction associated with essential hypertension has been documented in peripheral and coronary macro- and microcirculation and in renal circulation. The mechanism responsible for this alteration appears to be the activation of an alternative pathway involving cyclo-oxygenase, which reduces the NO availability through production of oxidative stress (Katusic & Vanhoutte 1989;Taddei et al. 2001). The NO-system may interact with other peptides such as endothelin-1 (see below) to regulate the vascular tone (Schiffrin 1999a). NO inhibits the production and vasoconstrictor activity of ET-1, which was found to be impaired in essential hypertension (Taddei et al. 1999).

2.9.2.4 NOS inhibitors

Nitric oxide synthase can be blocked by L-arginine analogues or the endogenous competitive inhibitor, asymmetric dimethylarginine (ADMA) and by NOS antagonists, such as N-nitro-L-arginine methylester (L-NAME) and NG-

monomethyl-L-arginine (L-NMMA). It was found that these substances can inhibit NO production and consequently endothelial cell proliferation (Babaei et al. 1998). ADMA is a naturally occurring amino acid that circulates in plasma, is excreted in urine, and is found in tissues and cells (Vallance et al. 1992a;Vallance et al. 1992b). It has aroused interest because it inhibits nitric oxide synthase (NOS) and therefore can cause considerable biological effects, particularly on the cardiovascular system. Several recent studies have suggested that plasma concentrations of ADMA provide a marker of risk for endothelial dysfunction and cardiovascular disease (Cooke 2000). ADMA inhibits all three isoforms of NOS and is approximately equipotent with L-NMMA (MacAllister, Whitley, & Vallance 1994). It is a non-selective inhibitor of NOS, causing elevation of blood pressure, vasoconstriction and impaired endothelium-dependent relaxation, and increases endothelial cell adhesiveness (Achan et al. 2003). Furthermore, there is evidence that basal NO is reduced in hypertension and that the vasoconstrictor response to L-NMMA is reduced in untreated hypertension (Calver et al. 1992). Recently, a study showed that OSAHS may be associated with an elevated level of ADMA, which could be reversed by CPAP therapy (Ohike et al. 2005).

2.9.3 Endothelin-1

Endothelins are 21-amino-acid peptides encoded by three genes, and produced by the endothelium of blood vessels and also in many other tissues (Schiffrin 1999b;Schiffrin 2001). Endothelin-1(ET-1) is an endothelium-derived peptide with powerful vasoconstrictor and mitogenic properties, which is a paracrine or autocrine regulator of vascular tone (Schiffrin 2001;Yanagisawa et al. 1988). It has two receptors, ETA and ETB, and acts on adjacent endothelial or smooth muscle cells.

ET-1 has a short half-life, because it is rapidly cleared from the circulation, yet it has a sustained effect on vascular tone. Physiological low oxygen tensions (PO2 $\leq$ 30mmHg) increase endothelin-1 secretion from cultured human endothelial cells four- to eightfold above the secretion rate at ambient oxygen tension (Faller 1999). In addition, hypoxia increases endothelin-1 transcript within 30 minutes of exposure and persists for 48 hours, although this is reversible after re-exposure to normoxia (Kourembanas et al. 1991). Increased levels of endothelin-1 have been demonstrated in conditions associated with endothelial dysfunction, such as atherosclerosis, hypercholesterolaemia (Haak et al. 1994), and cigarette smoking (Haak et al. 2004). In human essential hypertension, circulating levels of ET-1 have been reported to be elevated by some, but not all investigators. ET-1 is overexpressed in African-American hypertensive patients compared with white Caucasian patients (Ergul et al. 1996). Schiffrin et al. have found enhanced expression of ET-1 gene in resistance arteries in severe human essential hypertension (Schiffrin 1999b). Another study found that salt-depleted, salt-sensitive hypertensive patients with blunted renin responses had enhanced catecholamine-stimulated endothelin levels (Elijovich et al. 2001).

The role of ET-1 in the development of hypertension in OSAHS patients is not clear. It has been suggested that ET-1 is not elevated in these patients (Grimpen et al. 2000), in contrast with earlier findings by Phillips et al. (Philips et al. 1999), who found that sleep apnoea elicits increases in blood pressure and endothelin-1, with a decrease in both after overnight CPAP treatment. However, it would be very difficult to be certain about these findings since ET-1 is rapidly and locally degraded.

2.9.4 Tissue Plasminogen Activator (TPA)

Tissue plasminogen activator (TPA) is a protein released by endothelial cells. It activates the reaction where plasminogen is converted to plasmin. Plasmin is a potent fibrinolytic enzyme that degrades all proteins. TPA regulates the fibrinolytic activity of blood in balance with another protein called plasminogen activator inhibitor-1 (PAI-1). PAI-1 is also produced by endothelial cells and helps in the regulation of fibrinolysis, serving as the primary inhibitor of TPA (Raitakari & Celermajer 2000). PAI-1 could be enhanced by low oxygen tension in contrast to TPA, which is decreased by hypoxia (Pinsky et al. 1998). This mechanism plays an important role in the suppression of fibrinolysis. In prospective studies, high concentrations of TPA antigen in apparently healthy men may be associated with an increased risk of subsequent myocardial infarction and stroke (Ridker et al. 1994). So far, few studies have found a relationship between sleep apnoea and fibrinolytic activity. Nevertheless, Rangemark et al. found that PAI-1 was greater in OSAHS patients compared with healthy controls. They concluded that low fibrinolytic activity may represent a confounding pathophysiological mechanism behind the high incidence of myocardial infarction and stroke in sleep apnoea patients (Rangemark et al. 1995) .

2.10 Conclusion

Thus, when the present investigations were being designed, evidence was accumulating that OSAHS was associated with hypertension. Some comparative data also suggested that baroreceptor function might be abnormal in patients with OSAHS, although no randomized placebo-controlled studies of the effect of the treatment of OSAHS on baroreceptor or endothelial function had been conducted. Thus, studies were designed to address these issues.

Chapter 3

Methodology of Measurement in Baroreflex Study

3.1Introduction

The data reviewed in Chapter 2 show that OSAHS is associated with altered baroreceptor (Tkacova et al. 2000). However, they are epidemiological or before-and-after treatment studies which cannot exclude other reasons for the association. To determine whether OSAHS caused altered baroreceptor and whether this was reversed by effective treatment, we performed a randomized placebo controlled trial of CPAP therapy on baroreceptor in patients with OSAHS.

3.2 Methods of Baroreflex Measurement

In humans when blood pressure increases, there is a linear relationship between the systolic blood pressure of each cardiac cycle and the subsequent pulse interval (Pickering et al. 1972).The assessment of this relationship assumes a positive correlation between a stimulus and a response, in this case, a change in blood pressure and a change in the pulse interval (PI). It therefore reflects the ability to alter vagal activity in relation to the blood pressure in a reciprocal manner. BRS is diminished in several diseases, most notably hypertension (see Chapter2).

The measure of an intact baroreflex is the degree of change in the heart rate or sympathetic stimulation for a given unit change in blood pressure (Bertinieri et al. 1985a). It can be quantified as the response of the cardiovascular system to the application of an external stimulus, mechanical or pharmacological (La Rovere et al. 1998) in standardized conditions. However, there is an alternative way of evaluating baroreflex modulation, by identifying spontaneous episodes of consecutive beats in

which progressive increases or decreases in systolic blood pressure (SBP) are followed by concordant R-R interval (RRI) changes (Bertinieri et al. 1985a). These sequences have been shown specifically to reflect the baroreflex activity, hence their relationship is used as an index of the sensitivity of the cardiac baroreflex (Parati, Di Rienzo, & Mancia 2000;Parlow et al. 1995).

These sequences, in physiological conditions, are often interspersed with SBP changes that are not coupled with reflex changes in R-R modulation. A new index has been proposed to quantify baroreflex activation in modulating HR, the baroreflex effective index. The arterial baroreflex induces beat-to-beat changes in pulse interval in response to only around 21% of all the ramps in blood pressure (Di Rienzo et al. 2001). Baroreflex effective index quantifies the number of times the baroreflex is clearly effective in driving the sinus node, whereas baroreflex sensitivity quantifies the power of the reflex when this drive is effective (Di Rienzo et al. 2001).

Baroreflex sensitivity can also be quantified by spectral analysis, which is expressed by the gain of the transfer function relating changes in blood pressure to coherent changes in the R-R interval. This approach is based on the coherent relationship between the SBP and R-R interval, which oscillate in the same frequencies in the power spectrum, controlled by the baroreflex. De Boer and colleagues indicated that this frequency region lies between 0.04 and 0.35 Hz (de Boer, Karemaker, & Strackee 1985b). This region is divided into very low frequency (VLF), low frequency (LF) – which avoid the possible influence of respiration – or high frequency (HF) region, which is more driven by respiration (de Boer, Karemaker, & Strackee 1985a). In a simplified approach, BRS can be determined by power spectral analysis of heart rate period (1/HR) and BP using Fast Fourier Transformation (FFT)

and expressed as the square root of the total power of the pulse interval frequency divided by the total Power of the BP frequency, which is known as the alpha coefficient

BRS= √ (power R-Interval/ power SBP).

A characteristic of the alpha approach is that it models the BP-RRI relationship by assuming that all the RRI power in the considered frequency band is generated by the baroreflex. For statistical reasons, it is accepted that this is correct if the SPB-PI coherence in the frequency band considered for the estimate is > 0.5 (Pagani et al. 1988). These methods for computing baroreflex gain assume linearity of the relationship between changes in blood pressure and R-R interval, which is defined as the interval between adjacent normal QRS complexes resulting from sinus node depolarization (1996b).

Respiration modulates the influence of the baroreflex on cardiac vagal motor neurons: inspiration decreases and expiration enhances the cardiac vagal responses to baroreflex activation (Eckberg & Orshan 1977). Hyperventilation impairs baroreflex modulation of HR and sympathetic nerve traffic, and increased BP during hyperventilation does not elicit any reduction in heart rate (Van De Borne et al. 2000). A change in the respiratory pattern might itself affect respiratory sinus arrhythmia. This is discussed in greater depth in Chapter 8 on Reproducibility and Chapter 9.

3.3 Recruitment

3.3.1 Patients

Consecutive patients who attended the Sleep Centre at Edinburgh Royal Infirmary and who met the inclusion criteria were approached. Patients were contacted in

person at the Centre, by telephone, or by writing to them, explaining the objectives of the study and giving an information sheet. If the patient agreed, he or she was randomized for treatment order, using a balanced block design, and a date sent for the CPAP titration study.

Patients were told that the study was to compare two different potential treatments for OSAHS, one was standard treatment, and the other was new. The standard treatment was CPAP and the new treatment was treatment with capsules. They were told that the capsules might affect upper airway tone and therefore reduce the number of breathing pauses. Thus, the placebo was actively sold to our patients as a possible active treatment. Patients were told that we wanted to look at the effects of both treatments on their blood pressure in a crossover design. This design had the approval of the Lothian Ethics of Medical Research Committee (See appendix 3).

Healthy subjects were also recruited as a ccontrol for the baroreceptor sensitivity study (section 3.13).

3.3.2 Recruitment Criteria

Inclusion criteria

1. AHI ≥ 15 by full polysomnography or apnoea/hypopnoea per hour ≥ 25 with limited sleep study.
2. Epworth sleepiness scale >11
3. Aged between 18 and 75 years

Exclusion criteria

1. Living > 50 miles from the sleep centre
2. Taking any medication that affects blood pressure or might augment the autonomic nervous system, including all anti-hypertensive drugs, cardiac

(anti-anginal and heart failure) drugs, anti-depressant drugs, anti-psychotic drugs, and steroids.

3. Any co-existing medical disorder that might affect blood pressure, for example, diabetes.
4. Patients with cardiopulmonary diseases, for example, COPD, IHD, CHF or severe asthma.
5. Driving Problems as a result of sleep apnoea (to avoid delaying effective treatment).

All patients gave written informed consent to participate in the study, which was approved by the Lothian Ethics of Medical Research Committee.

3.4 Screening

The main recruitment was by identifying patients at the Friday clinical case review meeting of the department of sleep medicine and directly from the sleep clinic. The screening process was performed by reviewing patients' case notes, referral letters and the sleep questionnaires for patients and their bed partners followed by direct questioning of patients about their past medical history. Chapter 4 gives details of the screening results.

3.5 Desaturation index

From diagnostic polysomnography, the desaturation index (DI) was usually calculated. Each PSG trace is reviewed manually and any parts with oxygen saturation artefact edited out. DI was also calculated from the limited home sleep study. The number of 4% oxygen desaturations is counted during the total sleep time (wake periods were excluded). This total number was then divided by the total sleep

time in minutes and multiplied by 60, giving the number of events per hour slept, which is known as the desaturation index.

3.5.1 Measurement

Non-invasive technologies allow the continuous monitoring of oxygen saturation of arterial blood (SaO2). Pulse oximeters use spectrophotoelectrical principles to determine SaO2 from a two-wavelength light transmitter and receiver placed on either side of the pulsating vascular bed. These devices are sensitive only to tissues that pulsate. The sites of monitoring may include finger, ear and nose, which are all recommended for measurement. In the sleep laboratory, the finger oximeter is mainly used as part of a full polysomnography or limited home sleep study. The finger should be kept straight, because significant bending of the digit can restrict the ability of the devices to detect pulsatile flow, the absence of which precludes SaO2 determination.

3.5.2 Body Measurements

Body length measurements were made using a dressmaker's standard measuring tape. The waist was measured at the umbilicus, the hips were measured at the level of the iliac crests, and the neck was measured at the level of the crico-thyroid membrane. Height was recorded from a fixed standard scale, to allow calculation of body mass index (BMI = weight/height^2 in kg/m^2). Weight was measured using the standard Sleep Centre scales (Seca, Germany), which are calibrated annually. Measurements were recorded in centimetres (cm) and kilograms (kg) respectively. Patients were weighed on each visit to ensure that there was no important change in their weight during the study.

3.5.3 Ambulatory Blood Pressure Monitoring (ABPM)

ABPM was performed using a lightweight microprocessor, which is carried on a belt or shoulder strap, connecting to the arm cuff via a rubber hose (Ultralite ABPM, 90217, Space Labs Medical Ltd). The ABP monitor measures 2.8 x 11.4 x 8.6 cm and weighs 347g. It has a four-digit liquid crystal display on the front panel, a manual start/stop button, and attachment for the BP cuff. The rear panel contains a serial communications port and the on/off switch. The data can be transferred to a personal computer via a PC interface for formal analysis. The monitors are carried in pouches, which are worn with either a belt or a shoulder strap. The monitors can be programmed to carry out various functions such as displaying cuff pressure at each bleed step, and displaying systolic, diastolic, mean arterial pressure and heart rate at the end of each cuff deflation. They continue to bleed to 40 mmHg rather than stopping at the diastolic value. Although they can bleep before and after each recording is taken, the bleep was disabled during this study. The machine takes Korotkoff sound one for the systolic and five for the diastolic pressure. The monitor bleeds air in discrete steps (approx. 4 mmHg), and uses the oscillometric method of BP determination. The monitor measurement ranges are heart rate 40–180 beats per minute; systolic BP 70–280 mmHg; diastolic BP 40–200 mmHg; and mean arterial BP 60–240 mmHg. The microprocessor can be programmed to take recordings at intervals of 6 to 120 minutes. In this study, the monitor was programmed to record at intervals of 30 minutes for 48 hours, day and night. It takes approximately 30–50 seconds to make a recording. After any failed attempt, the monitor is programmed to try a further recording one minute later. The cuff pressure inflates to 170 mmHg initially, then to 30 mmHg above the previous systolic recording. The

microprocessor is programmed to distinguish between pressure signals, patient movement and respiratory artefact. The pressure transducer channel automatically zeros before taking a recording. The cuff is inflated with an automatic pump, and the bleed rate is also controlled via the microprocessor. The device contains a real-time clock, which is recalibrated with each monitor initialization. The monitor is initialized before being fitted to each patient. This ensures that the time clock is correct and allows the patient's identification data to be stored with the collected data. The microprocessor auto-edits aberrant results. For safety, the measurement cycle is limited to 180 seconds, and the absolute maximum pressure of the cuff is 310 mmHg.

The patient was fitted with a cuff of appropriate size to the non-dominant upper arm with appropriate instructions on how to readjust the cuff when necessary. Patients were asked to continue their normal daily activities and to record these in a diary. The monitor recorded systolic BP (SBP), diastolic BP (DBP), heart rate (HR) and the mean arterial pressure (MAP).

3.6 Measures of Daytime Sleepiness

3.6.1 Epworth Sleepiness Scale

Appendix 1

The Epworth sleepiness scale (ESS) is a self-administered eight-item questionnaire (Johns 1991a) that was developed as a tool to measure subjective sleepiness in adults. The scale has been found to be a reliable for measuring persistent daytime sleepiness (Johns 1992a) and can show differences with treatment and time (Hardinge, Pitson, & Stradling 1995). It does not, however, differentiate between different causes of sleepiness (Johns 1993;Johns 1994b). The patients were asked to

score themselves on a self-rating scale totalled from 8 ratings of 0–3, each depending on how easily they would fall asleep in a specific situation. The overall score ranges from 0–24: the higher the score, the sleepier the individual. All the patients were asked to fill the ESS at the beginning of the study and at the end of each treatment limb.

Table 3.1 ESS

Situation	**Chance of dozing**
Sitting reading	
Watching TV	
Sitting, inactive in a public place (e.g. a theatre or a meeting)	
As a passenger in a car for an hour without a break	
Lying down to rest in the afternoon when circumstances permit	
Sitting and talking to someone	
Sitting quietly after lunch without alcohol	
In a car, when stopped for a few minutes in the traffic	

3.6.2 FOSQ (Functional Outcomes of Sleep Questionnaire)

Appendix 2

This is a sleep specific questionnaire developed to reflect the impact of sleep disorders and excessive sleepiness on the activities of daily life. It is a self-administered questionnaire consisting of 30 questions, focusing on five different aspects: general productivity, social outcomes, activity level, vigilance, sexual

relationships, and intimacy. The optional questions on intimacy and sexual relationships were excluded in this study as is our custom (Faccenda 2000;Teixeira, Faccenda, & Douglas 2004). The questionnaire consists of 26 questions, each with a four-point scale. The results are processed to give a mean-weighted item score for each of the four sub-groups, which when added, produce a global score. The lower the score, the more dysfunctional is the individual secondary to sleepiness. This questionnaire has been validated for use with OSAHS patients and control population and can be used to determine how disorders of excessive sleepiness affect patients' abilities to conduct normal activities and the extent to which these abilities are improved by effective treatment of sleep disorders (Weaver et al. 1997). At the end of each treatment FOSQ were scored and all the data were analysed with SPSS.

3.8 CPAP Night

All patients underwent a CPAP titration study, regardless of their subsequent treatment randomization, which was in random order done by a third person. Before the CPAP night, patients were usually educated about sleep apnoea/hypopnoea syndrome and CPAP treatment. This teaching was usually done on their baseline visit. They were fitted with an appropriate CPAP mask and then had a 20-minute CPAP trial in a darkened room. This mini-trial provided the patient with an opportunity to ensure that the mask fitted correctly and gave him or her the feeling of positive pressure treatment before the overnight titration study.

Titration was conducted using either Autoset or Autoset T CPAP machines (ResMed, Australia) under the supervision of experienced specialist sleep nurses. Titration usually commenced between 10:30 and 11:00 p.m. and ended around 7:00 a.m. the

next morning. The Autoset machine was downloaded and the 95th centile pressure was usually taken.

3.9 Treatment

Patients were randomized to receive either CPAP or placebo for one month in a crossover study.

3.9.1 CPAP

Following the CPAP titration night, a fixed-pressure CPAP machine with memory (ResMed or O'Sullivan) had been initialized to the 95th centile CPAP pressure. Patients were taught how to use the machine and the mask. Patients were given a number to call if they had any problem during the month and they were contacted once. On a few (5) occasions, some patients had a home visit to change mask. At the end of the treatment trial, the CPAP machine memory was downloaded and this provided data on night-to-night use and the overall compliance.

3.9.2 Placebo

The study used a crossover design to assess effects of CPAP on blood pressure and baroreflex. CPAP was compared with oral treatment with capsules. Patients were told that these capsules might improve the upper airway tone. When this study was started, "sham" CPAP had not been fully developed and tested and there were concerns about its effect on sleep disruption and oxygenation. An oral placebo has been used in previous OSAHS studies. These studies were mainly testing blood pressure changes (Faccenda et al. 2001c) and daytime function (Engleman et al. 1998). The placebo was given in a sealed white medicine bottle containing 35 white capsules labelled "trial medication". Patients were instructed to take one capsule with water before going to bed. They were also given a contact number to call if they had

any problems during the month. I usually asked the patients to bring back any remaining capsules as an indication of compliance.

3.10 The Protocol

All patients underwent a full night in the sleep laboratory for CPAP titration study, using an automated pressure setting device (as described above). The following morning, the patient was randomized by a research nurse using a balanced block design to receive either CPAP or an oral capsule for the first limb, and crossed over after a month to the alternative treatment for the second limb. The patients were told that the capsules might improve the tone in the upper airway. The CPAP units used for home therapy were Sullivan V Elites (ResMed Australia). At the end of the treatment period, data were downloaded to obtain a real-time record of the time when the patient was using the device at the appropriate pressure (the compliance). The patients were fitted with the ambulatory blood pressure monitor for 48 hours at the end of each limb. The monitors were fitted at around 6 p.m. in all patients, and programmed to measure blood pressure every 30 minutes for 48 hours. Patients were asked to abstain from caffeine-containing products for the whole 48 hours. Data of the first 24 hours were discarded to allow for acclimatization, and the analysis was performed only on the second 24 hours of monitoring. Thus, data was accumulated during 27 days from commencing that treatment, so that the risk of carry-over effects of the other treatment was reduced. All the data were manually checked for artefacts by an independent observer, who did not know the treatment status of the patient. At the end of each treatment period, the patient was invited for the baroreflex sensitivity test. Weight and height were measured to allow the calculation of the body mass

index (kg/m2). The ESS and FOSQ were completed at the start of the study and at the end of each limb.

3.11 The Measurement of Baroreflex Sensitivity

3.11.1 Finapres

General principles and theory of the technique

A method of non-invasively measuring the complete arterial waveform was developed by Dr Jan Penaz. The technique uses the concept that if an externally applied pressure (via bladder in the cuff) is equal to the arterial pressure at all times (instantaneously), the arterial walls will be unloaded (zero transmural pressure) and the arteries will not change in size. Since the arteries under the cuff are not changing in size, the blood volume, which is only contained in the arteries at these pressures, will not change, so the photoplethysmogram will be constant at some value.

The measurement technique works by taking the above concept in reverse. First, the constant blood volume has to be determined, as represented by the photoplethysmogram, which becomes a "set point" for a servo loop. It means that as the measured photoplethysmogram varies from the set point, so the servo valve is driven to increase or decrease the cuff pressure as required to maintain the measured photoplethysmogram at the set point value. The pressure in the cuff will be driven to equal the arterial pressure throughout each pressure cycle. This cuff pressure can be measured with an electronic pressure transducer and the resulting signal displayed as the arterial pressure. Setting and maintaining the set point is critical for an accurate blood pressure measurement by this technique. The Finapres blood pressure monitor uses two methods to determine the set point. The first method, known as the start-up adjustment, is an automated process similar to what Dr Penaz did manually, and is

used to establish an initial approximate set point. The second method, known as the servo self-adjustment, provides fine-tuning of the set point and corrects for slow moving physiological changes occurring in the finger and arteries under the cuff.

The Finapres is an accurate method of monitoring short-term changes in BP (Silke & McAuley 1998), and can track the changes that occur during the Valsalva (Singer et al. 2001;Tank et al. 2001) and Müeller manoeuvres, atrial fibrillation (van den Berg et al. 2001), and OSAHS (Tkacova et al. 2000). Digital arterial pressure, however, differs from brachial artery pressure because of the hydrostatic effects of the hand position, and the normal changes in the BP along the vascular tree. Thus, the plethysmographic volume clamp is best used as an *index of change* in BP rather than of absolute pressure. Furthermore, the Finapres derived pressure has been shown to correlate well (r = 0.96) with invasive intra-arterial blood pressure monitoring during anaesthesia (Huang et al. 2000).

Description

I used the Finapres monitor (Ohmeda, USA) figure 3.2 that uses Penaz principle. It provides continuous measurement of finger arterial pressure. It has a diagonal shape with a screen that displays measurements. It has also four soft keys by which the operator can change the displayed page. These keys give control on other functions such as alarm, and calibration.

Cuff Application

This device uses a pneumatic servo-controlled cuff, which is inflated to keep the infrared absorption in the finger constant by the correction of a photoplethysmogram. The infrared absorption in the finger alters owing to the variation in blood volume in the finger; changes in cuff pressure are therefore required to keep the

photoplethysmogram constant, and these reflect changes in the intra-vascular pressure. The cuff must be properly applied to achieve accurate and stable blood pressure measurements. The middle finger is the one preferred for the cuff, although other fingers can be used if no pulse can be obtained from the middle one.

3.11.2 Procedure of monitoring

An appropriately-sized cuff was attached to the middle finger of the left hand. Patients were allowed to acclimatize with the equipment running, and rested unstimulated for 30 minutes before any recordings are made. The patient's hand was at the level of the heart, and the patient was instructed not to move the hand or the finger with cuff while recording. The servo-self adjust (in-built calibration mode) was disabled on the Finapres device during each 5-minute test run. It was then reactivated. If there was more than a 5 mmHg difference in the readings, the test was re-run. Data for the whole time were recorded; however, the analysis was conducted only of the five-minute time blocks. Three sets of recordings, lasting 5 minutes each, were taken for every patient during both visits.

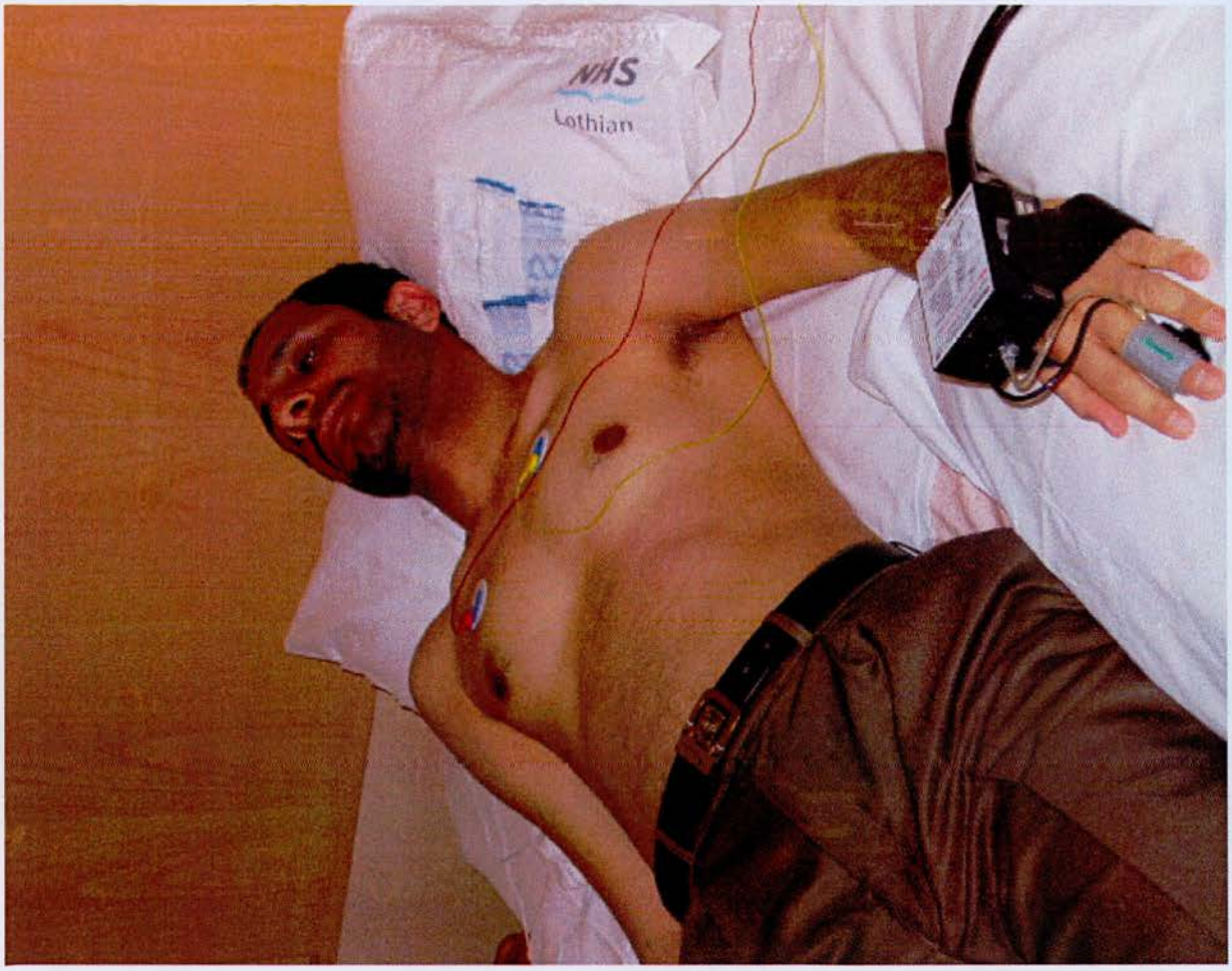


Figure 3.1 Example of BRS testing

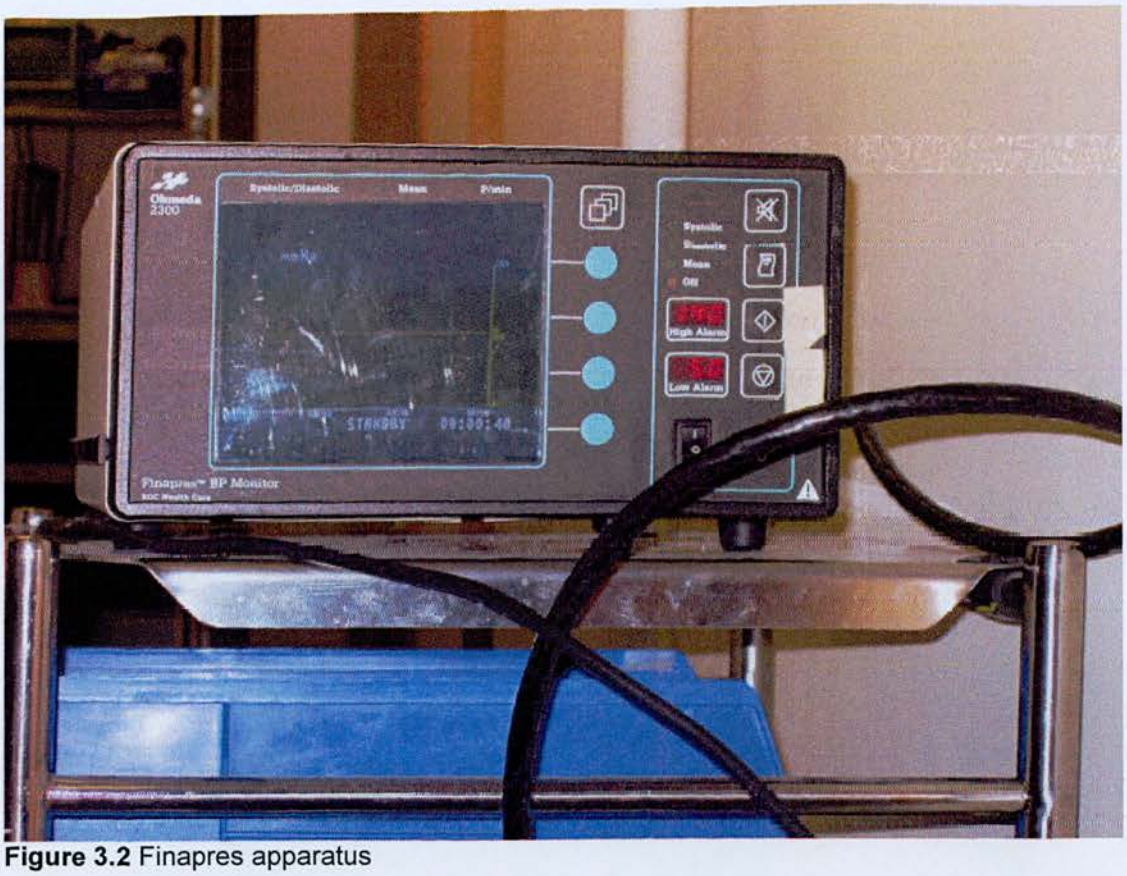


Figure 3.2 Finapres apparatus

3.12 Baroreceptor Reflex Sensitivity

The patients were asked to abstain from alcohol, smoking and caffeine for a minimum of 12 hours prior to attending for the study. All baroreceptor studies were performed between 8 a.m. and 12 p.m. The patients lay supine in a quiet, dimly lit soundproofed room and were left unstimulated. Electrocardiographic recordings were taken from chest leadII. The skin was prepared using an exfoliating gel and wiped with alcohol to ensure good contact before the electrodes were placed. Beat-by-beat blood pressure was recorded using the Finapres. Data from the ECG and Finapres were recorded on a PC, using a special montage in the Compumedics software, in which there were two recording channels, one for the ECG and another one for the Finapres. Data from these files were backed up onto optical disks.

Patients took part in this study at the end of each treatment limb before crossing over to the alternative treatment. Data were analysed using SPSS to compare the different treatments.

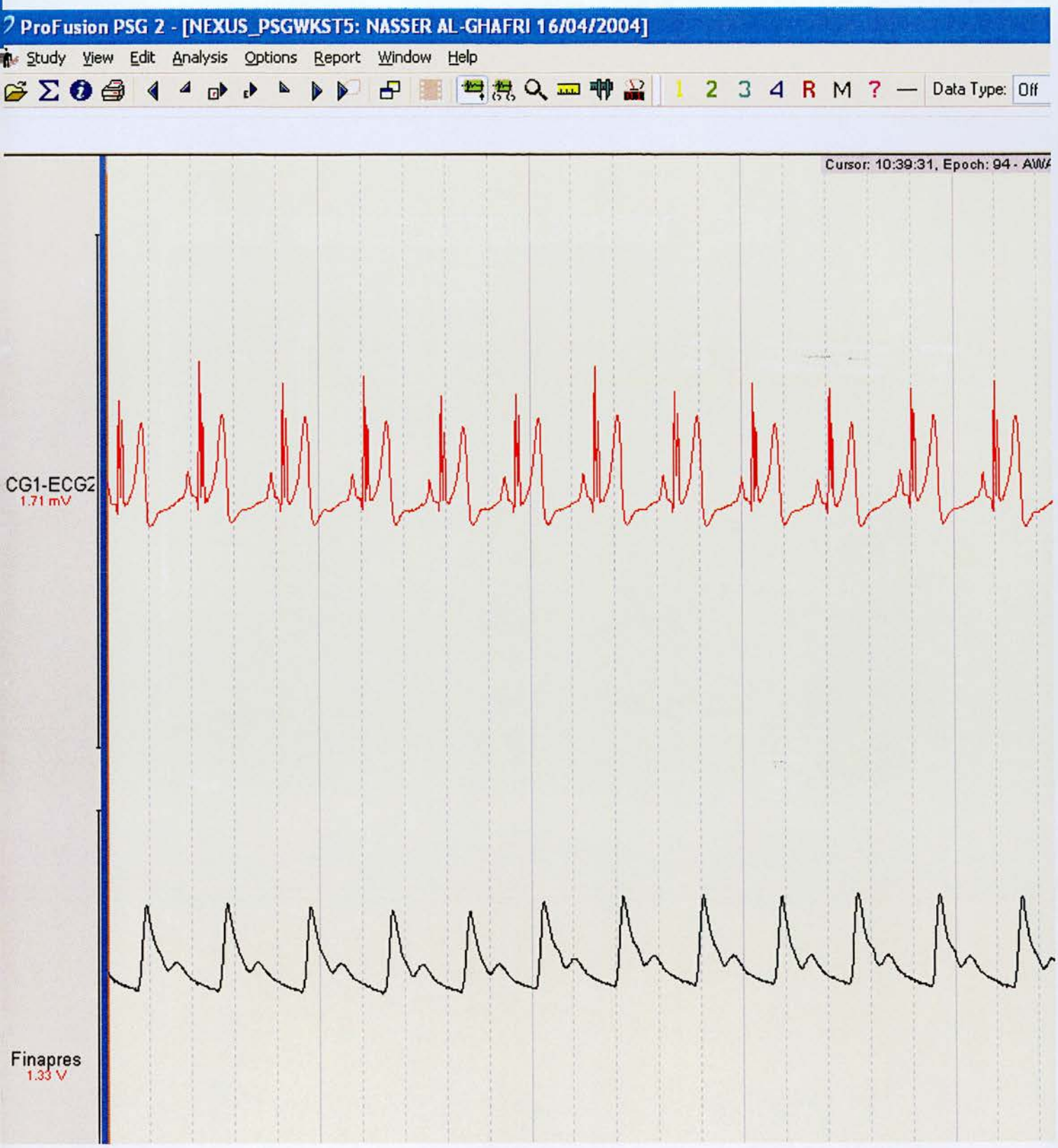


Figure 3.3 Example of baroreflex recording. The upper tracing represents two ECG chest leads and lower tracing is the pulse pressure measured by the Finapres.

3.13 Controls

Ten healthy subjects were recruited as controls for the baroreceptor sensitivity test. Recruitment was done by advertising in the University of Edinburgh and Edinburgh Royal Infirmary e-mail and messenger system. OSAHS was excluded by using a sleep questionnaire for the participants and his/ her partner with ESS < 5. Loud snorers were also excluded. The rest of the exclusion criteria were the same as above (Section 3.3.2). The BRS study was done once for each subjects and blood pressure was measured twice. The rest of the procedure was as above (Sections 3.8 & 3.9).

3.14 Data Analysis

The Finapres data were moved to an EDF file and then processed by a custom-written programme. The programme was written by a computer expert to analyse baroreceptor sensitivity, using both the sequential analysis method and the spectral analysis method.

3.14.1Sequence Method

The programme computed the linear regression between the sequences of systolic blood pressure values and the related pulse intervals which had a coefficient of determination (r^2) of the regression greater than 0.85. The programme determined the difference between each two consecutive systolic blood pressure and pulse intervals. It identified sequences with following characteristics:

systolic blood pressure increased by at least 1 mmHg in each of three or more blood pressure waves, and pulse interval increased by at least 4ms/beat in each of three or more cycles; and (2) systolic blood pressure fell by at least 1 mmHg during each of three or more blood pressure waves, and pulse interval decreased by at least 4 ms/beat (Bertinieri et al. 1988).

3.14.2 Spectral analysis method

Power spectral analysis is a frequency domain technique that enables the cross spectrum between BP and pulse interval to be quantified in amplitude or gain, phase and coherence. Power spectral analysis was performed on the R-R interval and SBP data, using an autoregressive algorithm. The following components were considered:

- Very low frequency (VLF) in the band below 0.04,
- Low-frequency (LF) power in the 0.04–0.15 Hz band, and
- High frequency (HF) power in the 0.15-0.4 band.

Coherence between the R-R interval and SBP was assessed by cross-spectral analysis. If it was more than 0.5, then power spectral analysis was performed on the RR interval and SBP data using autoregression. The alpha index was computed as the square root of the quotient of the R-R interval and SBP spectral powers in the LF and VLF bands.

Before starting the analysis, the calibration and the start and end time of each five-minute block were determined. The analysis was then done in two stages. First, any ectopic beats and artefacts were removed. Then each five-minute block of recording was analysed using the two methods. Then, all three 5-minute blocks of one visit were added together and the average was taken. The data were then saved in a text file before moving it to Excel, where further analysis was performed, and the data were transferred to SPSS for Windows for final analysis.

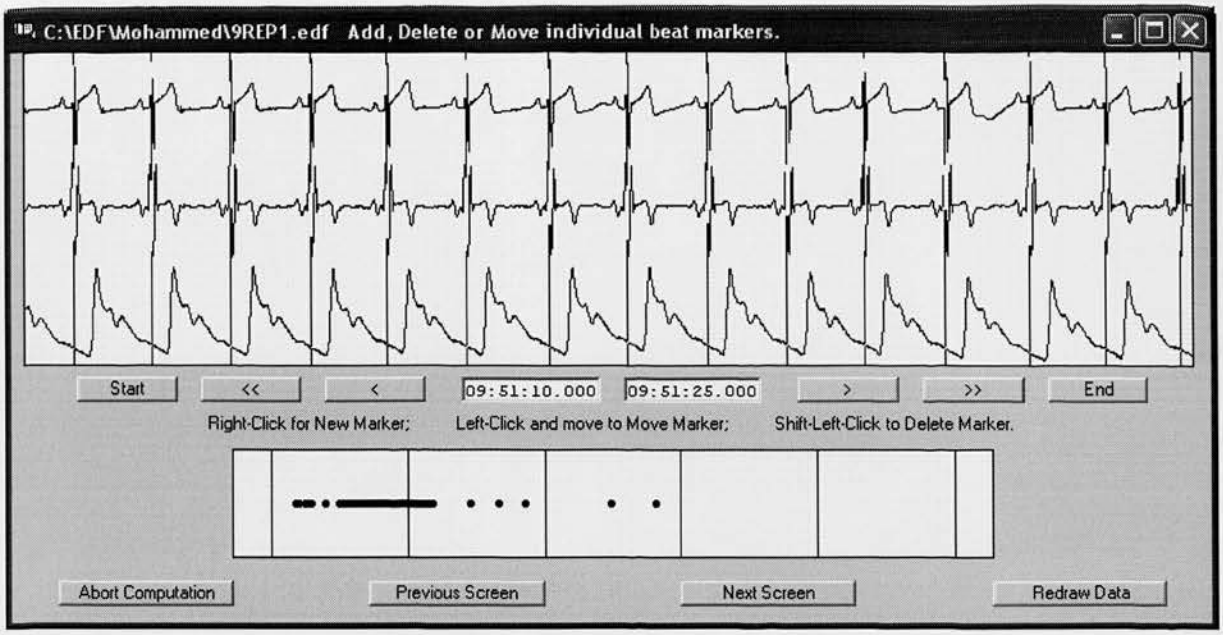


Figure 3.4 Processing of the ECG and pulse pressure with sequential analysis in which there is coordination between R- and the following systolic peak.

3.15 Measurement of Proteinuria and Creatininuria

3.15.1 Introduction

Normal subjects excrete less than 150 mg of protein per day in the urine (Cunningham 2002) and, with exception of orthostatic proteinuria, levels above this imply disease of the kidney or urinary tract. It has been recognized in recent times that even slight increases in the urinary albumin excretion can be a valid predictor of premature morbidity and mortality from cardiovascular diseases (Schrader et al. 2006;Wang et al. 2006). In addition, increases in albumin excretion are an early marker of nephropathy in patients with hypertension.

3.15.2 Mechanisms of Proteinuria

Normal barriers to protein filtration are present in the glomerulus, which consists of unique capillaries that are permeable to fluid and small solutes but effective barriers to plasma proteins. The adjacent basement membrane and visceral epithelial cells are

covered with negatively charged heparan sulfate proteoglycans (Kanwar 1984). Proteins cross to the glomerular fluid in inverse proportion to their size and negative charge. Proteins with a molecular weight of less than 20,000 pass easily across the glomerular capillary wall (Larson 1994). Conversely, albumin, with a molecular weight of 65,000 Daltons and a negative charge, cannot normally pass. The smaller proteins that do pass are largely reabsorbed at the proximal tubule, and only small amounts are excreted. The pathophysiological mechanisms of proteinuria can be classified as glomerular, tubular or overflow. Glomerular disease is the most common cause of pathological proteinuria (Stone 1989). Several glomerular abnormalities alter the permeability of the glomerular basement membrane, resulting in urinary loss of albumin and immunoglobulins (Abuelo 1983). Glomerular malfunction can cause large protein losses; urinary excretion of more than 2 g per 24 hours is usually a result of glomerular disease (Larson 1994).

3.15.3 Measurement of Proteinuria

Traditionally, proteinuria is measured using 24-hour urine collections and expressed as grams per day (g/day). This has the advantage of averaging out protein excretion and is not therefore affected by normal diurnal variation in protein excretion. Testing spot urine samples for protein has been introduced to overcome the inherent problems of patient accuracy and reliability with 24-hour urine collections. The urinary albumin concentration is measured by radioimmunoassay. Under resting conditions, urinary creatinine excretion is relatively constant throughout the day. Thus to overcome the problems of timing of urinary collections, proteinuria in spot urine samples is expressed as the albumin: creatinine ratio.

3.15.4 Microalbuminuria

Dipstick analysis is used in most outpatient, and sometimes in inpatient settings, to measure semiquantitatively the urine protein concentration. Dipsticks can detect a protein content of > 200 μg/min and they react primarily to albumin, being relatively insensitive to globulins and Bence-Jones proteins. In the urine, albumin is normally present in concentrations of < 20 μg/min, and the range of 20–200 μg/min is referred to as microalbuminuria, which is therefore by definition not picked up by dipstick testing (Beetham & Cattell 1993). Microalbuminuria is therefore defined as a urinary albumin excretion rate of 20–200 μg/min. The albumin excretion rate (AER) is around 25 per cent higher during the day than at night. There is a good correlation between the morning AER and the albumin: creatinine ratio in the first urine sample of the morning. The advantage of spot urine samples is that almost all patients can provide a sample when they attend the clinic (the Sleep centre in our case). Provided that each urine sample is taken at the same time, and the patient's dietary intake is largely constant, then these samples are very useful in assessing patients over time. The advantage of measuring the albumin:creatinine ratio is that it eliminates the timing of urinary samples, which is important in calculating the albumin excretion rate. Microalbuminuria can be used as a predictor of cardiovascular events in diabetic as well as hypertensive and cardiac failure patients (Rowley et al. 2000;Wardle 1982). Similarly, microalbuminuria may occur in healthy subjects after exercise and during normal pregnancy (Davenport 2003).

3.15.3 Proteinuria in OSAHS patients

Few studies have investigated the relationship between OSAHS and proteinuria. Earlier studies have shown that proteinuria is not uncommon in obese patients with

OSAHS and that this could be reversible (Sklar & Chaudhary 1988;Sklar, Chaudhary, & Harp 1989). However, more recent studies have reported that proteinuria is weakly associated with OSAHS (Casserly et al. 2001;Chaudhary, Rehman, & Brown 1995;Iliescu et al. 2001)

3.15.4 Methods and Samples

Patients were asked to give a spot urine sample, in a standard urine container used by the Royal Infirmary of Edinburgh, on three different occasions: baseline, after CPAP and after placebo, morning time (09:00-10:00). The sample was processed in the biochemistry laboratory by experienced technicians, who performed a radioimmunoassay to determine the concentration of albumin and creatinine in the sample so that a ratio of albumin to creatinine excretion could be derived.

3.16 Statistics

I used Microsoft Office Excel (Microsoft Office Excel 2003) to do the primary analysis of the 24 hours ambulatory blood pressure values. The recording was divided in to 6 time intervals before exporting the data into SPSS for comparative testing. Excel was also used to draw graphs and for preliminary calculation of spectral and sequence analysis.

I used SPSS (SPSS version 12.0 for Windows) to carry out analysis of variance for the treatment comparison.

3.16.1 Statistical Tests used in this Thesis

The paired-Sample T Test was used to compare two variables for a single case. It computes the differences between values of the two variables for each case and tests whether the average differs from zero.

The Wilcoxon signed-rank test considers information about both the sign of the differences and the magnitude of the differences between pairs. It was used to test data that were not normally distributed.

The Independent-Samples T Test procedure compares means for two groups of cases. Ideally, for this test, the subjects should be randomly assigned to two groups, so that any difference in response is due to the treatment (or control) and not to other factors. Furthermore, in this analysis, we assumed non-equal variance

The One-Way ANOVA procedure considers a quantitative dependent variable affected by a single factor (independent) variable. Analysis of variance is used to test the hypothesis that several means are equal.

Chapter 4

Results: Treatment Effect on Blood Pressure

4.1 Introduction

Ambulatory blood pressure was measured as part of the main study, which also included an assessment of sleepiness, baroreflex measurement, and measurement of microalbuminuria. There was no power calculation performed and these are small numbers and therefore this has a bearing on the validity of the results.

4.2 Preliminary results

Ninety consecutive patients were invited to participate in this study, of whom 35accepted the invitation. Of the 55 patients who declined to participate, most did so because of work commitments, the remainder for social and personal reasons. Of the 35 patients who agreed to participate, 29 completed the study: 24 males and 5 females. Three patients withdrew at the beginning of the study because of work commitments and personal reasons; one withdrew during placebo treatment and 2 during CPAP treatment. In addition, 10 healthy control subjects (8 males and 2 females) were also studied to measure baroreflexes only.

4.3 Patients' Screening

For the baroreflex study, I screened 705 patients who attended the Sleep Laboratory at the Royal Infirmary of Edinburgh from November 2000 to October 2002. Only 90 patients were eligible and contacted and Figure 4.1 gives details of the screening process.

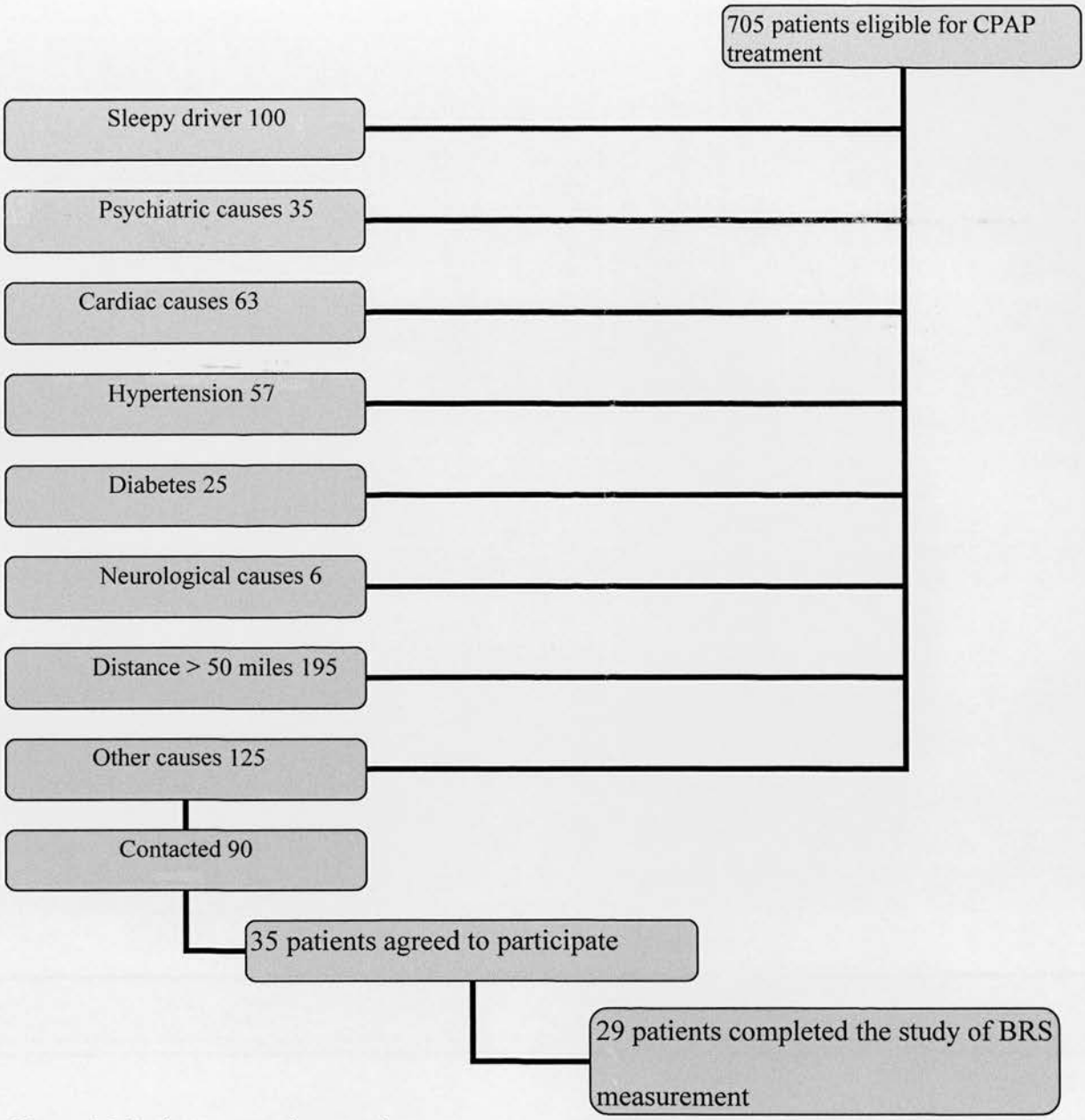


Figure 4.1 Patients screening results

The diagram shows that patient selection resulted in many patients being rejected, so that only 4.1% of the original number of patients took part in the study. Most of the patients were excluded because of co-morbidity. Hypertension and cardiac causes excluded about 17% of the total screened patients. Distance was another important reason for excluding patients from participation, since they had to be visited at least twice during the study and to come to Edinburgh at least 3 times. Sleepiness while

twice during the study and to come to Edinburgh at least 3 times. Sleepiness while driving was another major exclusion reason, since it was not ethical to include these patients because the placebo treatment could have exposed the patient to the risk of an accident. Other factors that affected the recruitment were that around 17% of the patients were booked for half-and-half clinical / CPAP titration and proceeded immediately to CPAP therapy. It was not ethical to delay their treatment. Finally, some patients were involved in other research studies.

4.4 Demographics

The demographics of the patients who completed the study are included in Table 4.1. Participants were compared with a random sample of 100 excluded patients, using the Student T- test.

Table 4.1 Demographics of participants and a random sample of 100 excluded subjects

	Participants		Excluded		P-value
	Mean	S.D.	Mean	S.D.	2-tailed
Age (yrs)	50	9	52	10	0.3
BMI (kg/m2)	33.8	6.6	32.9	7.7	0.6
ESS	13	5	15	5	0.2
Waist (cm)	110.3	17.3			
Hip (cm)	116.3	14.3			
Neck (cm)	42.8	3.8			
SBP (mmHg)	133	19			
DBP (mmHg)	85	13			

There was no significant difference between participants and excluded patients in age, BMI and ESS (P>0.2). Other patients' characteristics were calculated by non-parametric measures because the data were not normally distributed.

Table 4.2 non-parametric measures

		AHI	4%Desatuarion Dips	4% Desaturation index (dips/hours)	CPAP Use (hours/night)
N	Valid	29	26	26	29
	Missing	0	3	3	0
Median		44.3	97.0	19	4
Percentiles	25	31	27.5	4.1	2
	50	44.3	97.0	19	4
	75	59.4	202.3	38	6

4.5 Apnoea/Hypopnoea Index and Desaturation

Table 4.2 indicates that the median of AH1 was 44.3. The median 4% desaturation index was 19 (dips/hour). As expected, there was a significant correlation between the AH1 and the desaturation index ($P < 0.0001$).

4.6 CPAP Use

The median use of CPAP was 4 hours/night. Furthermore, there was no significant order effect of CPAP use. The mean use of patients starting with CPAP was 4.5 hours/night, and for those started with placebo, the mean was 3.4 hours/night.

4.7 24-hour Blood Pressure Results

4.7.1 Results of Intention to Treat Analysis

The result of data analysis was performed as intention to treat, irrespective of the compliance with treatment.

Table 4.3 24-hour Blood Pressure results of intention to treat analysis.

	CPAP		Placebo		**P-value**
	Mean	S.D.	Mean	S.D.	t-test
SBP (mmHg)	129	17	128	17	0.4
DBP (mmHg)	79	10	79	11	0.8
MAP (mmHg)	96	12	96	13	0.7
HR (bpm)	79	11	79	12	0.97

There was no significant difference in blood pressure in any parameters between the two treatments. The data were re-examined including only patients with more than ten 4% O2 desaturations/hour and CPAP usage > 4 hours per night (n=10). In this analysis, we obtained the results as shown in Table 4.4.

Table 4.4 24-hour blood pressure subanalysis results

	CPAP		Placebo		P-Value
	Mean	S.D.	Mean	S.D.	t-test
SBP mmHg	131	14	131	13	0.94
DBP mmHg	81	10	82	9	0.63
MAP mmHg	98	10	99	11	0.68
HR bpm	81	13	81	11	0.95

4.7. Diurnal changes in BP

Intention to treat analysis

Number of patients 29

Statistics

Blood pressure readings were grouped into six time intervals from 18:00 to 18:00 the following day. Comparison of the blood pressure values between treatments was made, with time of the day as the factor.

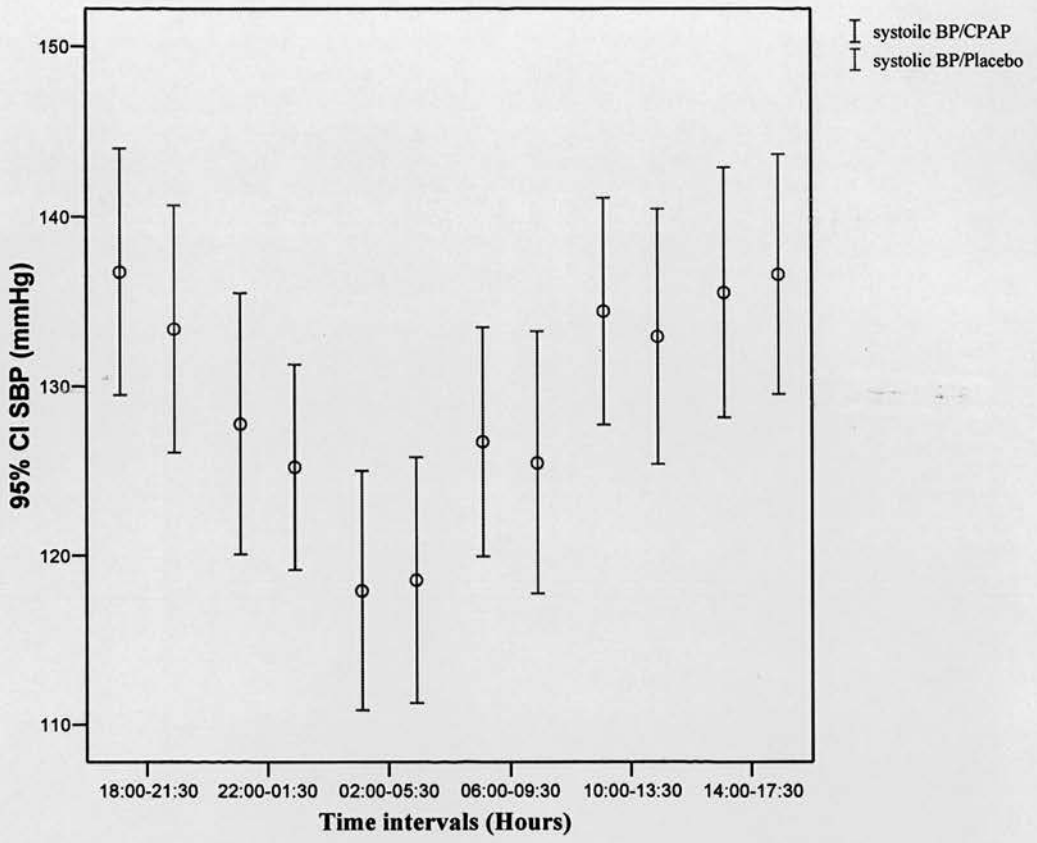


Figure 4.2 Diurnal changes in systolic BP and the effect of CPAP and Placebo treatments

I performed univariate analysis of variance for the diastolic blood pressure with the following results:

Table 4.5 ANOVA results of Diastolic BP

Dependent Variable: diastolic BP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	10355.6(a)	11	941.4	5.557	0.00
Intercept	2192170.3	1	2192170.3	12940.3	0.00
Treatment	212.4	1	212.4	1.3	0.3
Time	9999.8	5	1999.96	11.8	0.00
Treatment * Time	141.5	5	28.3	.2	0.97
Error	55903.8	330	169.4		
Total	2259862.0	342			
Corrected Total	66259.4	341			

a R Squared = 0.156 (Adjusted R Squared = 0.128)

There was no significant difference between the two treatments (CPAP and placebo).

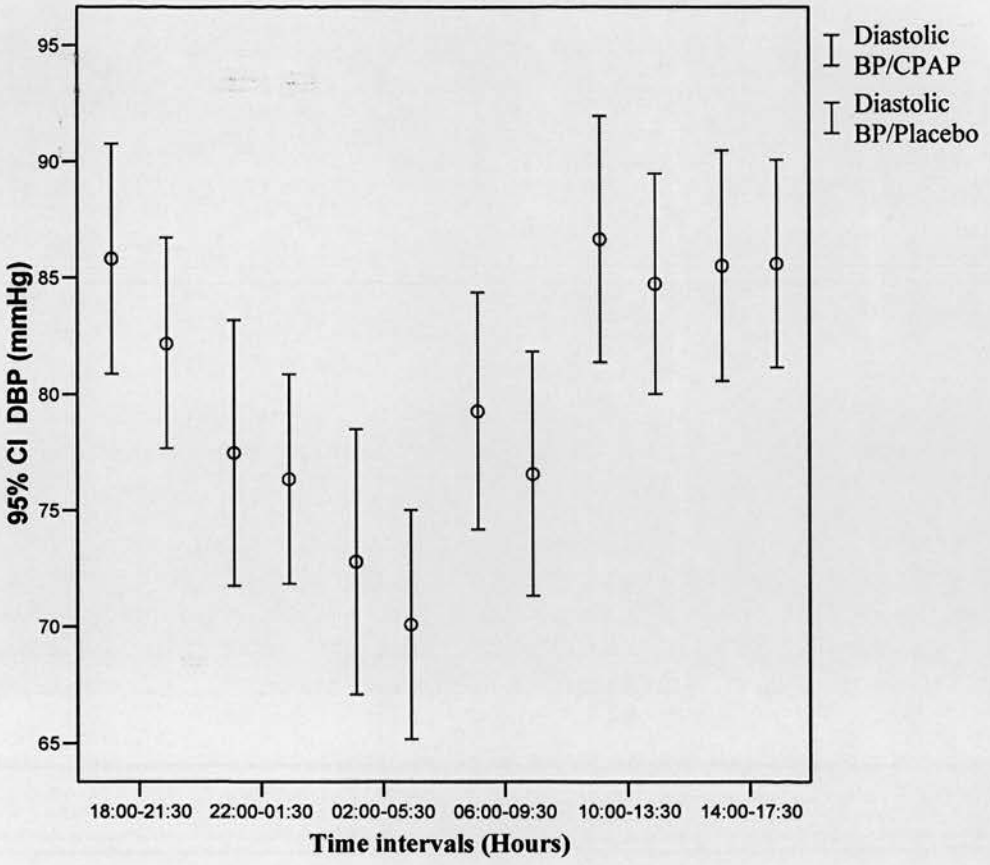


Figure 4.3 Diurnal changes in diastolic BP and the effect of CPAP and Placebo treatments

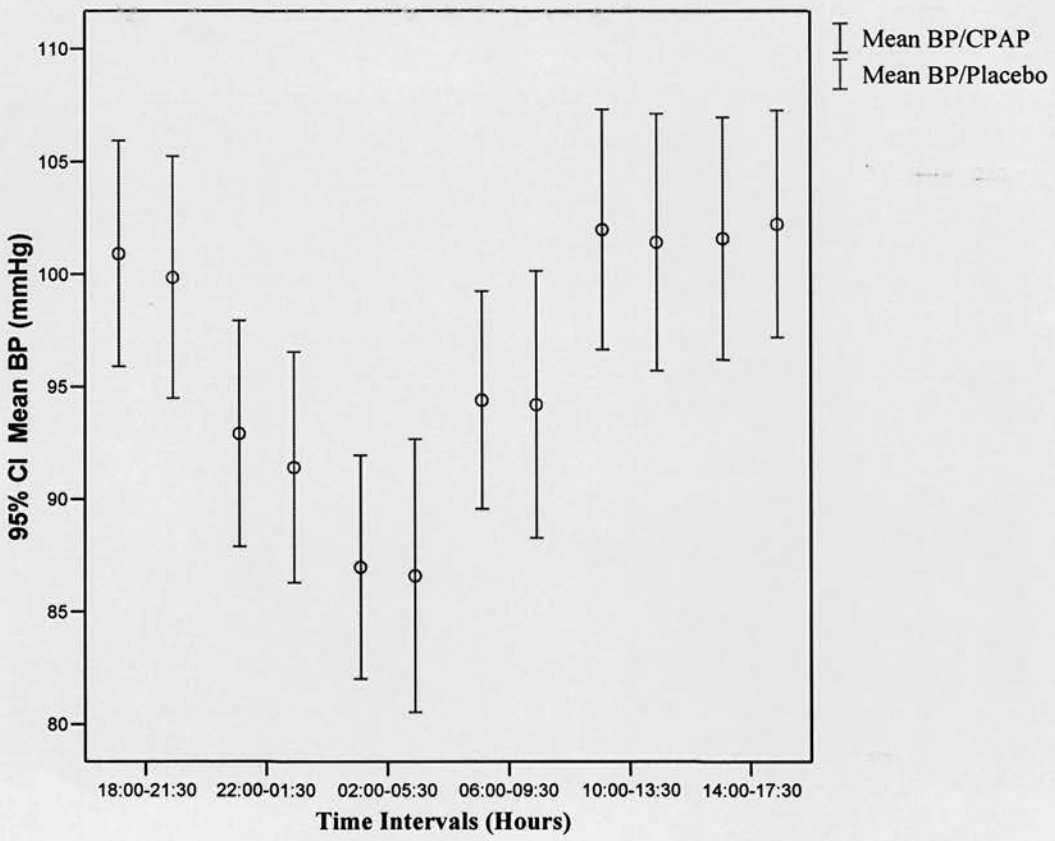


Figure 4.4 Diurnal changes in mean BP and the effect of CPAP and Placebo treatments

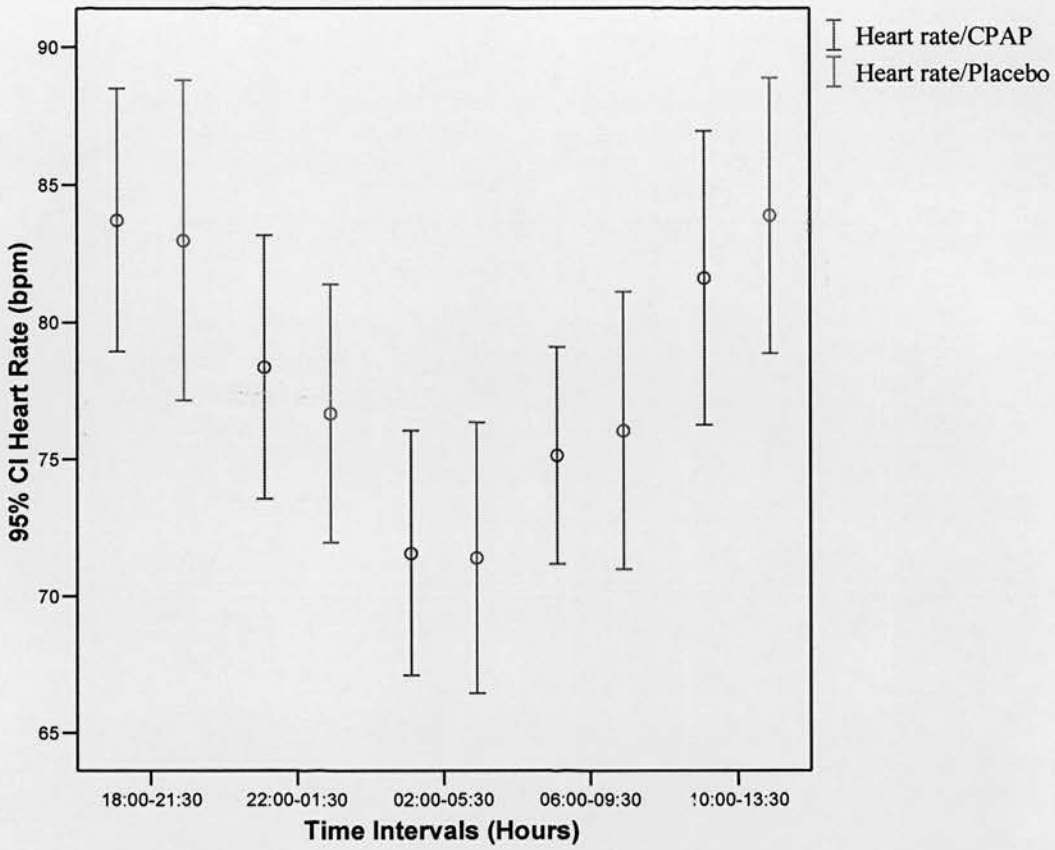


Figure 4.5 Diurnal changes in Heart Rate and the effect of CPAP and Placebo treatments

4.7.4 Diurnal Changes with Desaturation Index > 10 and CPAP Use > 4 hours

With sub-analysis of 24-hr BP monitoring, n = 10 in CPAP treatment and 9 in placebo patients

Table 4.6 Diastolic BP Univariate ANOVA-subanalysis

Tests of Between-Subjects Effects

Dependent Variable: diastolic BP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1822.6(a)	11	165.7	1.5	0.1
Intercept	635864.3	1	635864.3	5865.6	0.00
Treatment	157.6	1	157.6	1.4	0.2
Time	1608.8	5	321.8	2.96	0.02
Treatment * Time	56.2	5	11.2	.1	0.99
Error	9106.1	84	108.4		
Total	646793.0	96			
Corrected Total	10928.7	95			

a R Squared = 0.167 (Adjusted R Squared = 0.058)

Univariate ANOVA was performed with diastolic BP with no significant difference between CPAP and placebo treatment.

4.8 Linear Regression Analysis

The relationship between heart rate and desaturation index in patients treated with placebo was investigated with linear regression analysis. The correlation between heart rate and desaturation is significant in the period between (22:00-1:30) (R = 0.45, P = 0.02), and between 2:00–5:30 with R = 0.4, P = 0.04. The HR was positively correlated with desaturation index in these two periods, that is, HR increases with desaturation. See scatter plots.

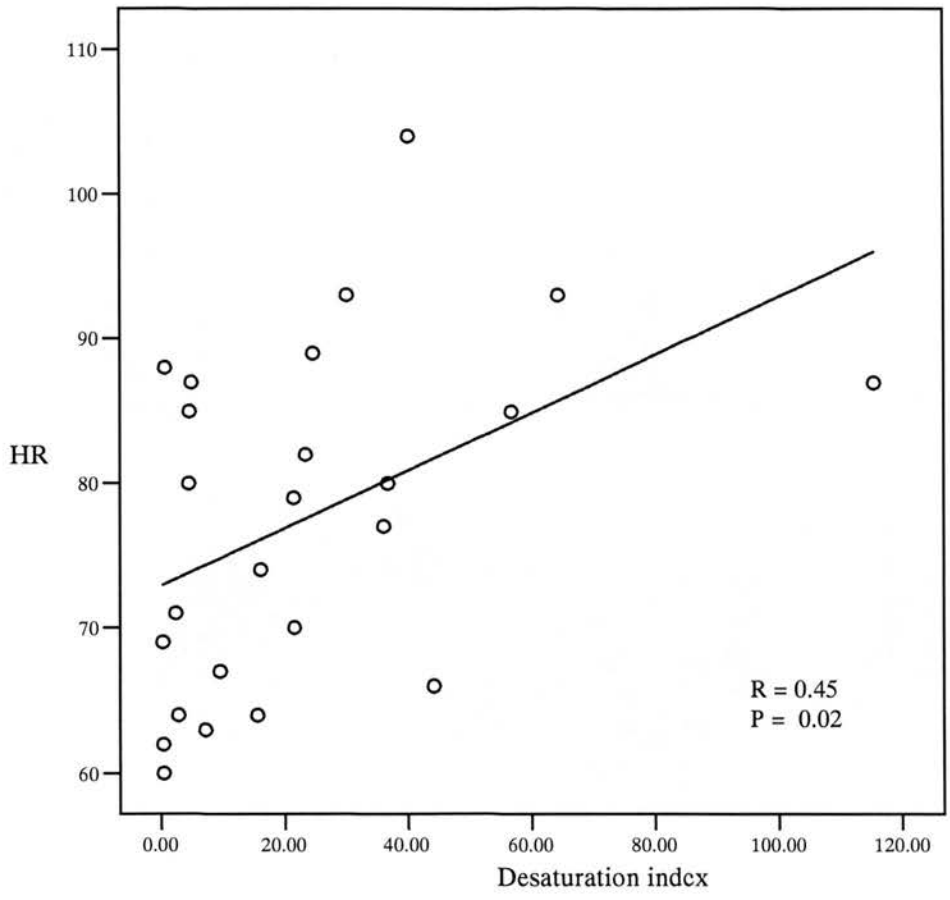


Figure 4.6 Heart rate (22:00–01:30)

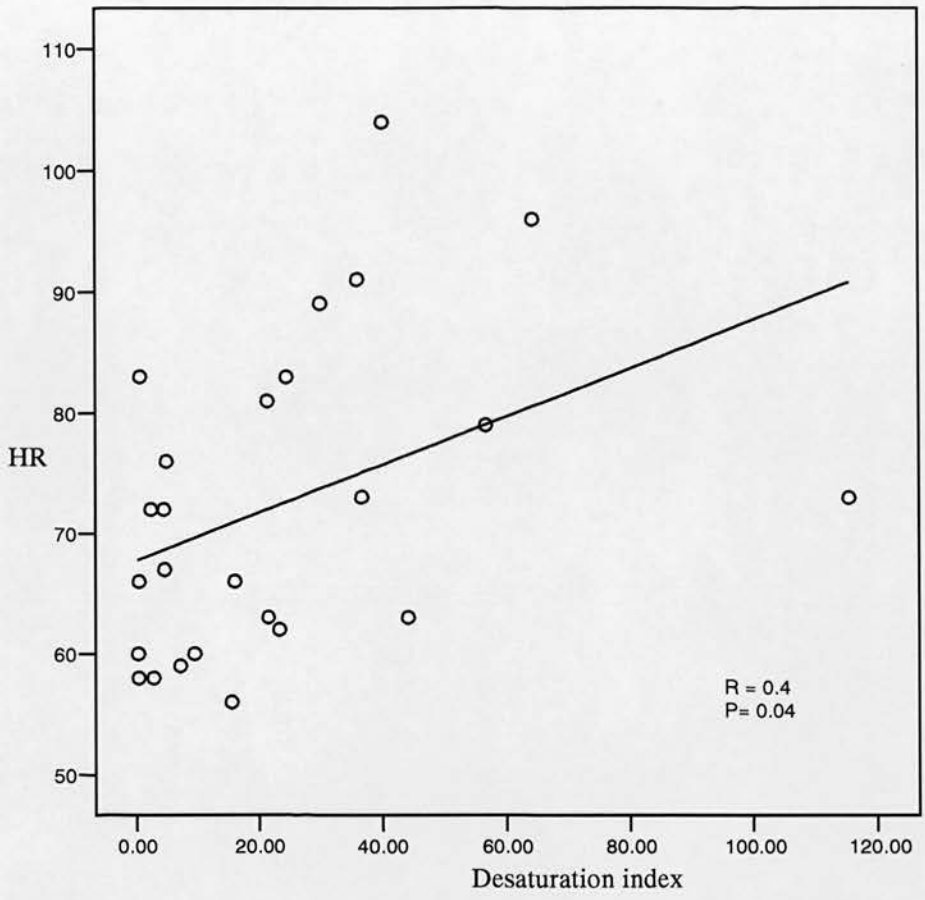


Figure 4.7 Heart rate (02:00–05:30)

4.9 Conclusion and discussion

There was no significant difference between CPAP and placebo treatment in all blood pressure measurements ($p>0.4$) in either intention to treat analysis or in the a priori sub-analyses of the compliant subjects or those with more than ten 4% desaturations per hour. Similarly, when the data were analysed by time of the day, there was no significant difference between the results after CPAP or placebo. Univariate ANOVA of Diastolic BP did not show any significant difference between CPAP and placebo in either intension to treat analysis nor in a priori sub-analysis of the patients who had severe AHI and were compliant. The regression analysis

indicates that hypoxaemia is a significant predictor for heart rate in OSAHS patients (p=0.02 & p=.04, r= 0.46 & 0.41 respectively) during the night-time (22:00-05:00). These findings suggest that hypoxaemia during sleep may have an effect on sympathetic activation of the heart (Narkiewicz et al. 1998c) which might lead to an increase in blood pressure. However, the correlation is weak and this study did not show any correlation between desaturation and any other measures of blood pressure. An alternative explanation for the relationship between hypoxaemia and heart rate would be that hypoxaemia contributed to arousal and thus indirectly increased sympathetic tone. The data in this study cannot distinguish between these two possible explanations.

As I discussed in chapter two, nasal CPAP treatment can reduce mean blood pressure. A non-randomised non controlled study suggested that one week of CPAP treatment reduced daytime and nocturnal blood pressure (Dimsdale, Loredo, & Profant 2000). A randomised crossover study found that one month of CPAP treatment reduced 24-hour diastolic blood pressure (Faccenda et al. 2001c), with greatest fall occurring between 2:00 am and 9:59 am (p=0.03). The reduction was more evident in patients with more oxygen desaturation and with CPAP use more than or equal 3.5 hours/night (see chapter 2 and chapter 12). In a randomised parallel study, nasal CPAP reduced mean ambulatory blood pressure by 2.5 mmHg. (Pepperell et al. 2002) The reduction in mean blood pressure was dependent on average nightly CPAP use and on 4% desaturation dips. Mean blood pressure fell by 4.9 mmHg in patients who used CPAP for longer than five hours, compared with no change in those who used CPAP for less than five hours. Patients whose blood pressure fell with CPAP had had more than 33 falls in arterial oxygen saturation of

greater than 4% every hour. In our study, we found that the median CPAP use was only four hours per night and the median 4% desaturation index was 19. These two factors may perhaps contribute to the lack of significant difference between CPAP and oral placebo treatments. In another study, ambulatory blood pressure was measured using finger Portapres for 20 hours (Becker et al. 2003). Here, 5.5 hours per night of CPAP use reduced mean blood pressure by 9.9 mmHg after 9 weeks of treatment. Our study only tested the effect of one month of CPAP treatment. A parallel, randomised, placebo-controlled trial (Campos-Rodriguez et al. 2006) showed that four weeks of CPAP therapy did not reduce blood pressure in hypertensive OSAHS patients who were undergoing antihypertensive treatment. The author concluded that four months of CPAP therapy was not enough to reduce the blood pressure in this group of OSAHS patients.

Chapter 5
Assessment of Sleepiness

5.1 Introduction

Patients' sleepiness was assessed throughout the study by means of the Epworth sleepiness scale (ESS) and the functional outcome of sleep questionnaire (FOSQ). All patients (n =29) completed the questionnaires on three different occasions: at the time of recruitment, and the end of each treatment period to assess the patients' sleepiness and response to treatment. Each questionnaire required 5 minutes to complete. . The mean baseline ESS was 13 ± 5. Other demographic details of the subjects have been discussed in Chapter 4.

5.2 Effect of Treatment

The ESS data show that patients improved with CPAP compared with placebo treatment (P =0.02, Paired t-test was used to calculate for the P value because it was a crossover trial) (Table 5.1, Figure 5.1)

Table 5.1 CPAP effect on ESS

	CPAP		Placebo		P-Value
	Mean	S.D.	Mean	S.D.	
ESS	9	5	12	5	0.02

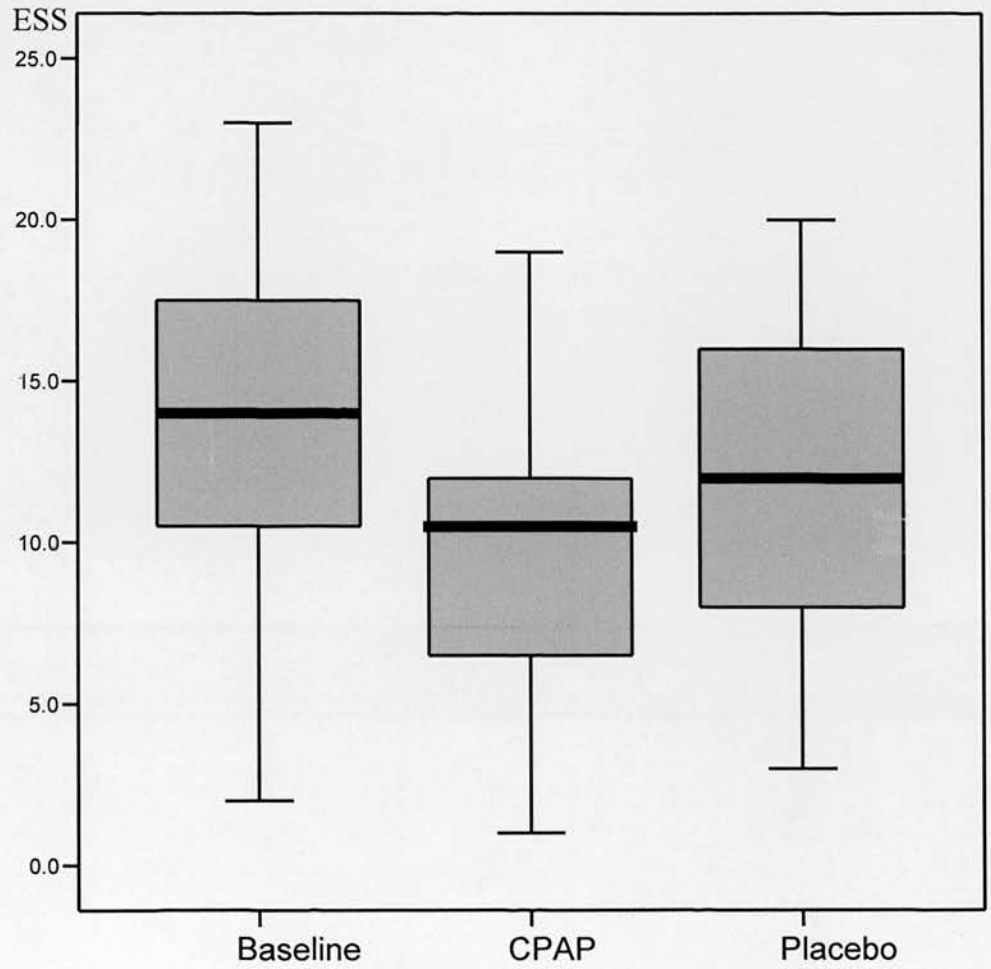


Figure 5.1 comparisons of baseline and CPAP and placebo effect on ESS

5.3 Treatment Effect on FOSQ

The effect of CPAP on total FOSQ score was not significant in comparison with placebo ($P > 0.05$). None of the four categories of FOSQ questionnaire was improved by CPAP treatment. There was no significant difference with any of the groups ($P > 0.2$) in all cases.

Table 5.2 Treatment effect on FOSQ

variable	Baseline		CPAP		placebo		CPAP v Placebo p-value
	Mean	S.D.	Mean	S.D.	Mean	S.D.	
FOSQ- GP	3.1	0.06	3.3	0.6	3.2	0.6	0.96
FOSQ-SO	3.1	0.9	3.3	0.8	3.3	0.9	0.5
FOSQ-AL	2.6	0.7	2.9	0.7	2.9	0.7	0.7
FOSQ-VG	2.6	0.8	3.0	0.7	2.8	0.6	0.2
FOSQ-total	11.4	2.6	12.5	2.5	12.2	2.6	0.96

Figure 5.2 summarizes the treatment effect between CPAP and placebo on total FOSQ scores, which also indicate that there was no significant difference, between CPAP (mean 12.5 ± 2.5) and placebo (12.2 ± 2.6), treatment compared with baseline for the score has marginally improved.

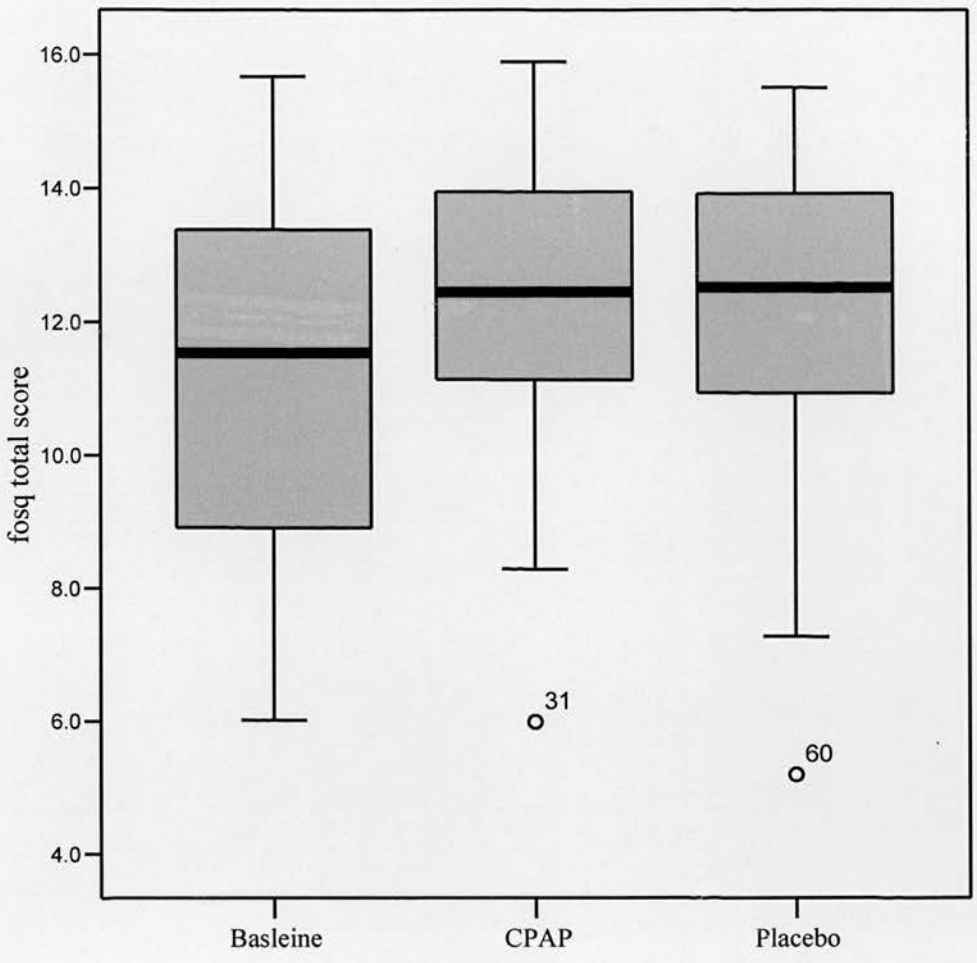


Figure 5.2 Treatment effect on FOSQ total score

5.4 Conclusion

We found that patients' subjective sleepiness determined by the ESS questionnaire improved after CPAP treatment (P=0.02). This result supports numerous other studies where OSAHS patients improve symptomatically with CPAP treatment (Engleman et al. 1994c;Engleman et al. 1997c;Hardinge, Pitson, & Stradling 1995;Jenkinson et al. 1999;Monasterio et al. 2001;Montserrat et al. 2001). However, the FOSQ results did not change after CPAP. This suggests the FOSQ may be a less sensitive tool. The result could also be affected by the fact that our subjects were

(mean 11.2 ± 2.6). This could limit the symptomatic improvement possible with CPAP. The relatively small number of subjects and thus low power may have also contributed to this negative finding. In a study of 48 patients by Engleman et al showed that ESS score was significantly reduced by CPAP treatment. FOSQ score was also improved with CPAP. (Engleman et al. 2002). Relatively low CPAP compliance may also contribute to the negative result of the FOSQ. Another study by Montserrat et al.(Montserrat et al. 2001) showed that OSAHS patients significantly improved after six weeks of CPAP treatment compared with sham CPAP. CPAP reduced ESS score significantly but not the total FOSQ score. However, there was significant difference in general productivity and vigilance. In another study (Monasterio et al. 2001), CPAP did not significantly improve FOSQ score in mild sleep apnoea patients after three months of treatment with trend of significance after six month. Nevertheless, CPAP reduced symptoms of sleep apnoea. The baseline score of FOSQ and ESS was 101 and 12.1 respectively. These results indicate that total FOSQ score may not improve statistically with short-term CPAP use (one month in our study), and longer period with good compliance of treatment is required to show the difference.

Chapter 6
Effect of CPAP Treatment on Baroreflex Sensitivity (BRS)

6.1 Introduction

The main aim of this thesis is to investigate the treatment effect of OSAHS on baroreflex sensitivity (BRS). When this study was started, studies indicated that BRS was impaired in OSAHS (Carlson et al. 1996;Tkacova et al. 2000), although none were adequately controlled. Thus, we investigated the effect of CPAP treatment on BRS in patients with OSAHS, using non-invasive methods.

6.2 Comparison between Patients and Controls

6.2.1 Demographics of subjects

We studied 29 patients with OSAHS (24 males and 5 females) and 10 controls (8 males and 2 females) whose characteristics were:

Table 6.1 Demographics of patients and controls

	OSAHS patients		Controls		P-Value
	Mean	S.D.	Mean	S.D.	t-test
Age (yrs)	50	9	45	12	0.3
BMI (kg/m2)	33.8	6.6	26.9	5.3	0.1
Waist (cm)	110.3	17.3	98.1	21.5	0.9
Hip (cm)	116.3	14.3	103.9	13.5	0.3
Hip/waist	1.1	0.11	1.1	0.14	0.8
Neck (cm)	42.8	3.8	39.1	6.6	0.7
SBP (mmHg)	133	19	112	11	0.2
DBP (mmHg)	85	13	75	8	0.9
ESS	13	5	5	2	0.03

There was no significant difference between patients (n = 29) and controls (n = 10) in any of these characteristics (Student t test) except that the controls had significantly lower Epworth sleepiness scores compared with the OSAHS patients (P = 0.03). However, the patients tended to have higher, though not significant, BMIs than the controls. There was no significant difference in hip/ waist ratio between the two groups. There were 10 smokers among patients and only one smoker among control subjects.

6.3 Comparison of BRS between Patients and Controls

Table 6.2 demonstrates that although control subjects tended to have higher BRS values than patients there was no significant difference between the two groups in

any of the measures of BRS (P=0.2, Student t test). Nevertheless, Figure 6.1 and figure 6.2 show the BRS values in patients and controls.

Table 6.2 Statistics: BRS measurements of patients and controls

	Controls		Patients		P-Value
	Mean	S.D	Mean	S.D.	2-tailed
BRS (sequential analysis) (ms/mmHg)	8.2	5.2	5.5	4.4	0.2
VLFBRS (ms/mmHg)	3.7	1.8	3.5	2.3	0.9
LFBRS (ms/mmHg)	7.3	4.1	6.5	5.8	0.7
HFBRS (ms/mmHg)	14.3	9.2	9.5	9.3	0.2
Alpha BRS (ms/mmHg)	10.8	6.2	8.0	7.3	0.3

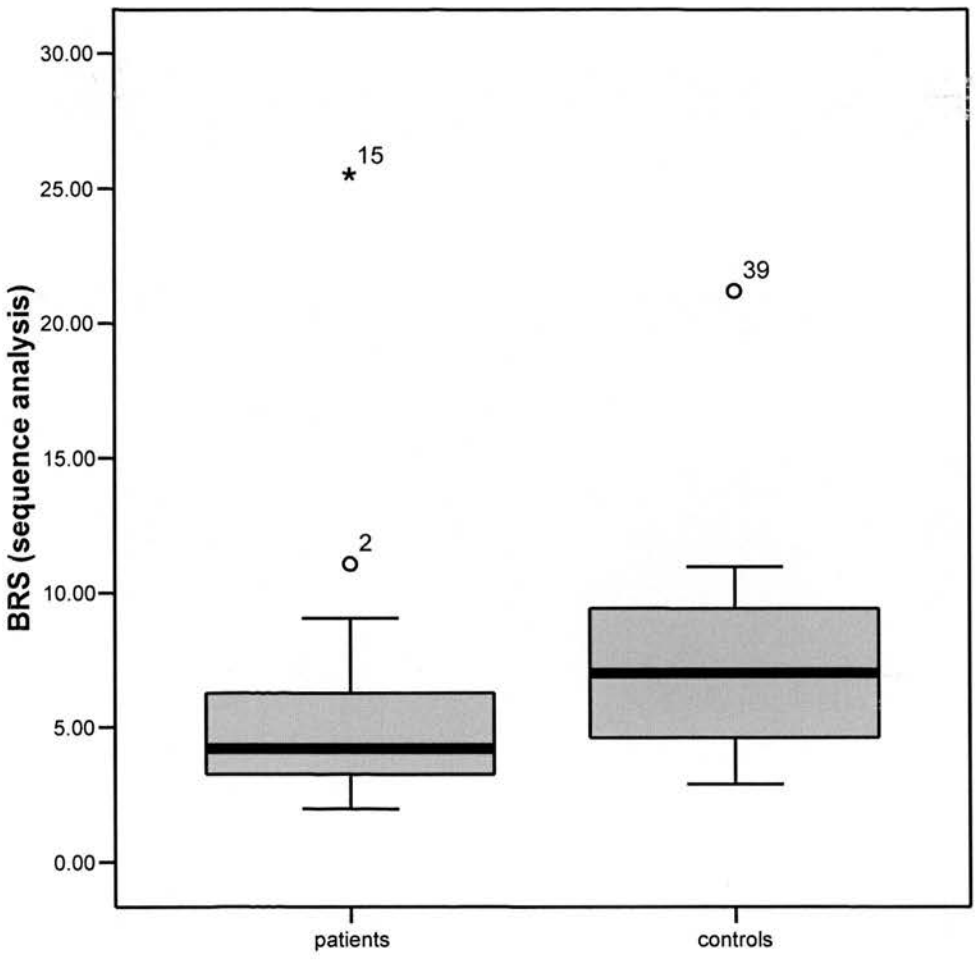
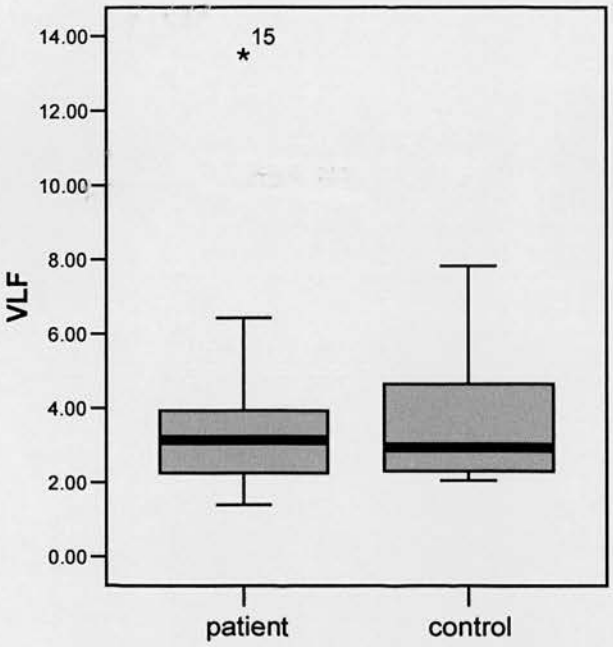


Figure 6.1 sequence analyses of BRS on OSAHS patients and healthy controls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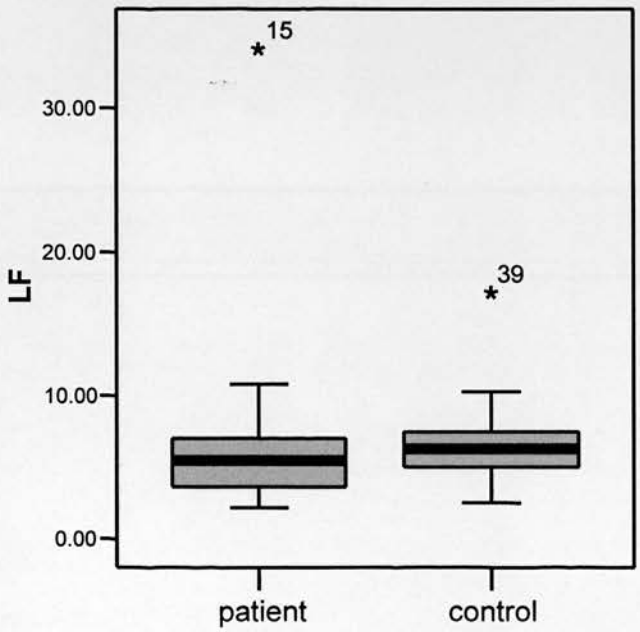
patient
control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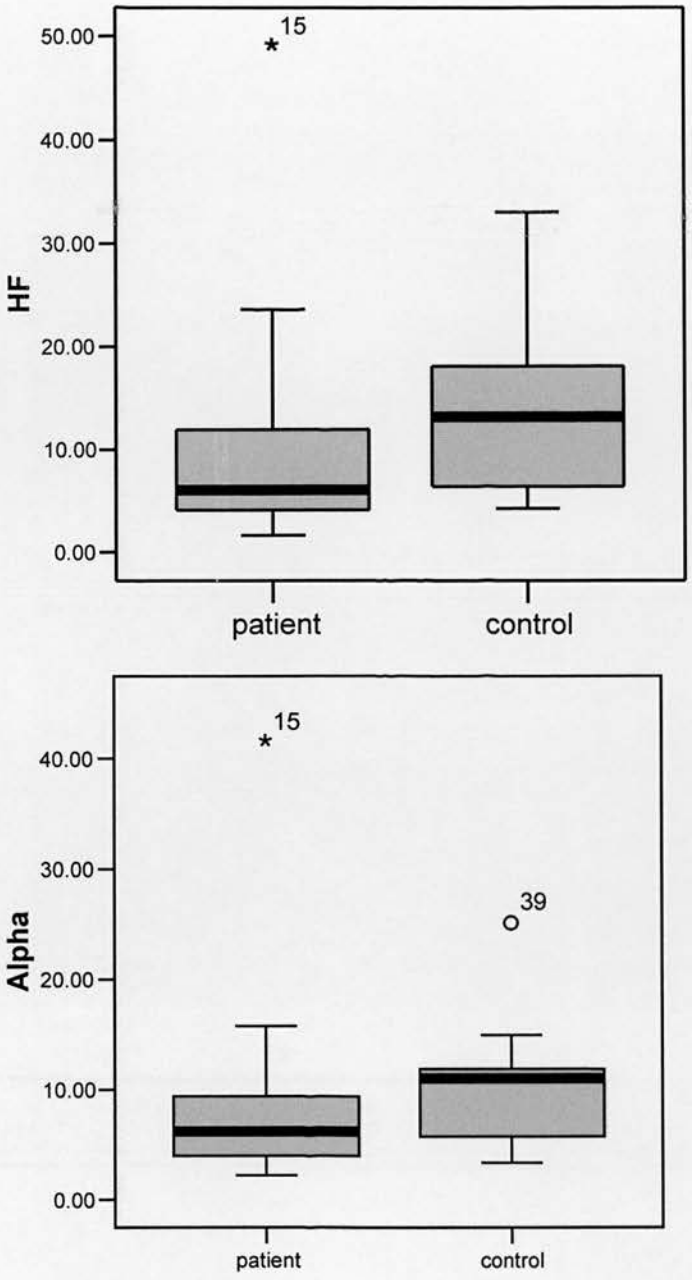


Figure 6.2 Spectral analysis of BRS in OSAHS patients and healthy controls

6.4 Effect of Treatment on BRS

There was no significant difference between the effect of CPAP and the placebo in this study. The initial calculation was done for all the patients who completed the study, that is, 24 males and 5 females. As shown in Table 6.3, there was no

significant difference between CPAP and the placebo in this study in any of the variables ($P > 0.1$).

Table 6.3 Treatment effect on BRS for all cases

	CPAP		Placebo		P-Value
	Median	Range	Median	Range	
BRS(ms/mmHg) (Sequence analysis)	4.8	1.7-14.7	4.2	2.0-25.6	0.9
VLFBRS (ms/mmHg)	2.5	1.5-5.8	3.1	1.4-13.5	0.5
LFBRS (ms/mmHg)	4.9	1.6-15.7	5.5	2.2-34.2	0.1
HFBRS (ms/mmHg)	6.5	1.9-24.3	6.0	1.6-49.2	0.97
Alpha BRS (ms/mmHg)	5.9	1.8-20.0	6.2	2.2-41.7	0.7

The Wilcoxon test was used to calculate these P values as the data were not normally distributed. In the a priori selected cases in which the 4% DI was more than 10 and CPAP usage was more than 4 hours/night (ten subjects), the BRS and other variables did not differ significantly between CPAP and the placebo either, as shown in Table 6.4.

Table 6.4 Subanalysis of BRS (4% DI > 10 & CPAP use > 3.9)

	CPAP		Placebo		P-Value
	Median	Range	Median	Range	
BRS (ms/mmHg)	5.0	1.7-6.6	4.2	2.0-9.1	0.8
VLFBRS (ms/mmHg)	2.3	1.6-4.1	3.0	1.5-4.5	0.2
LFBRS (ms/mmHg)	5.1	3.3-7.5	5.9	2.8-7.7	0.2
HFRS (ms/mmHg)	7.7	2.1-22.2	6.8	1.6-16.3	0.1
Alpha BRS (ms/mmHg)	7.0	2.7-13.6	6.4	2.2-11.0	0.6

The non-parametric Wilcoxon Test was used to calculate the P-Value

The graphs in Figure 6.3 show that there was no significant difference between CPAP and the placebo, even though the placebo gives higher BRS values than CPAP.

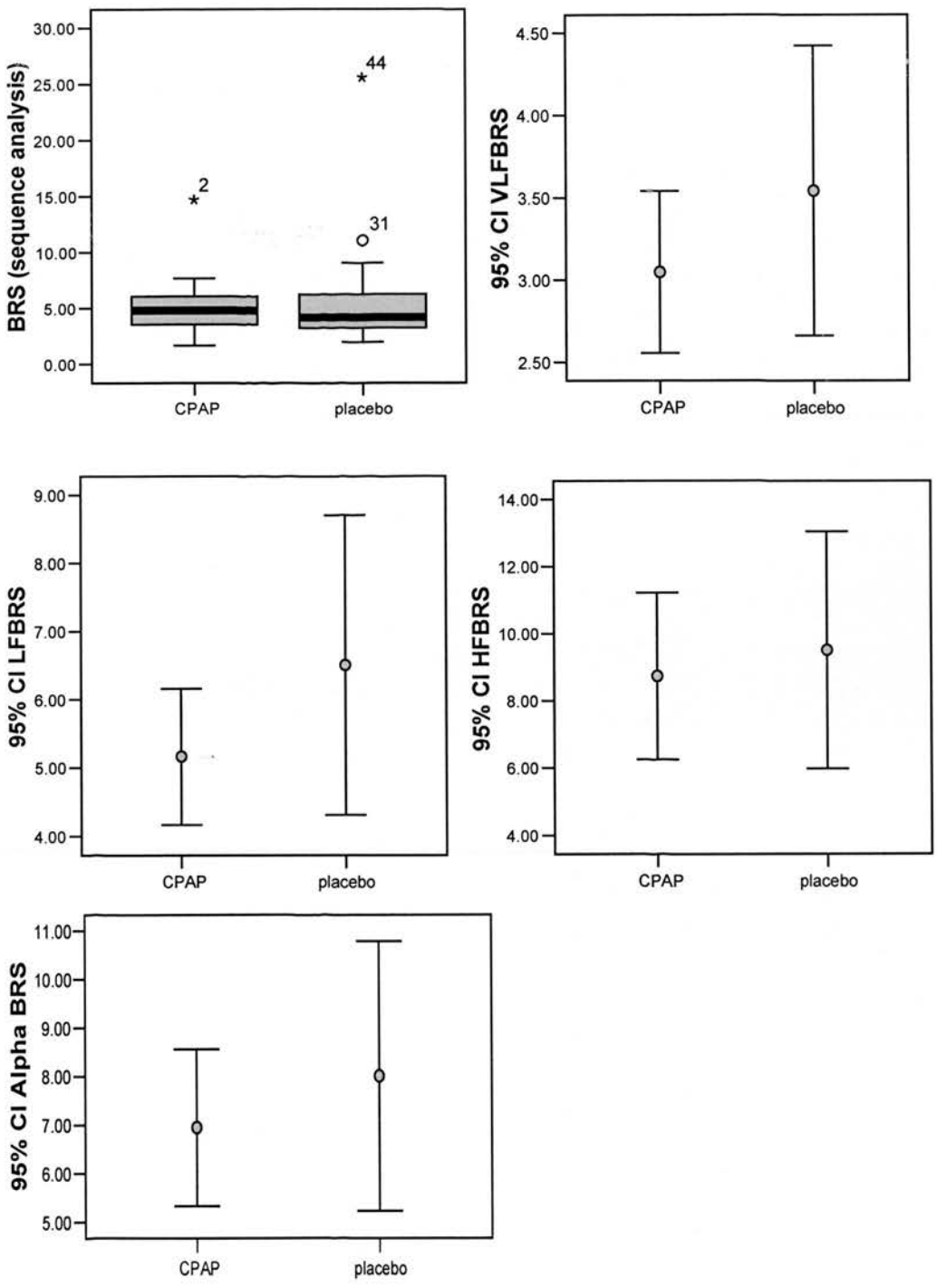


Figure 6.3 Treatment effect on BRS of OSAHS patients.

6.5 Conclusions

We found no significant difference between OSAHS patients and healthy subjects in the sequential domain analysis as well as the frequency domains of baroreceptor sensitivity ($P > 0.2$). This does not support other studies using similar comparisons. such as a study by Carlson et al. who found that baroreflex sensitivity is depressed in OSAHS patients compared with healthy controls (Carlson et al. 1996). They used a different technique to evaluate the baroreflex sensitivity. They quantified baroreflex sensitivity by changes in RR-interval and muscle sympathetic nerve activity, evoked by a reduction of blood pressure by sodium nitroprusside. Furthermore, in the present study, none of the baroreceptor sensitivity indices changed after CPAP therapy, even when data were analysed on the basis of a priori criteria designed to select a sub-group most likely to show changes by excluding the less-compliant and non-desaturating patients. The lack of changes in OSAHS patients either in comparison with normals or with CPAP might be due to:

1. there being no change in BRS with OSAHS
2. the relatively low power of the study or
3. The lack of reproducibility of the baroreceptor test used.

Studies have shown that baroreflex sensitivity in OSAHS improved after CPAP treatment (Bonsignore et al. 2002;Bonsignore et al. 2006;Logan et al. 2003;Tkacova et al. 2000). Cooper et al (Cooper et al. 2004), found that BRS was reduced by breathing with an inspiratory resistance in simulated obstructive sleep apnoea. Bonsignore et al studied (Bonsignore et al. 2006) the effect of CPAP treatment on

BRS for short periods (1-30 days). They assessed BRS by the sequence method using activation and deactivation of pulse intervals and systolic blood pressure. They found improved baroreceptor deactivation (downward sequencing) but not baroreceptor activation (upward sequencing) but no significant change after CPAP treatment in mean blood pressure or heart rate. Our study was more comprehensive in terms of measuring BRS using sequence as well as spectral analysis of R-R intervals and systolic blood pressure. Smoking may have an effect on sympathetic outflow in humans and consequently increase in blood pressure and heart rate (Mancia et al. 1997;Narkiewicz et al. 1998). In our study there were 10 smokers among patients and only one smoker among control that could have a bearing effect on the result. More potential explanations will be discussed in depth in the chapters on reproducibility and in the discussion.

Chapter 7
Validity and reproducibility of BRS measurement methods

7.1 Introduction

Even the standard method of assessing BRS, which involves the intravenous injection of a pressor agent such as phenylephrine(Smyth, Sleight, & Pickering 1969) has technical difficulties. There is a high test failure rate in patients with an attenuated BRS, and intra-subject reproducibility is poor even in normal controls. It is invasive and requires an intravenous injection. Importantly, phenylephrine has a negative effect on the sinus node (Schuessler et al. 1988). New methods to assess the BRS have been developed lately to overcome these problems, combined with the development of a non-invasive measurement of BP; these have resulted in simpler measures of BRS. The new methods, either the sequence method or the spectral analysis method, as described earlier, have been validated by comparing them with the previous phenylephrine technique; generally, the relationship between results obtained by the two methods was not impressive. The new approach was tested by Bertinieri et al., who recorded arterial blood pressure for 3 +/- 0.4 hour (mean +/- s.d) in 10 unanaesthetized, unrestrained cats. The recording was scanned by a computer to identify the spontaneous sequences of three or more consecutive beats in which SBP progressively rose and pulse interval progressively lengthened (type 1) or SBP progressively fell and pulse interval progressively shortened (type 2) (Bertinieri et al. 1985b). In this study, they found that all sequences had high correlation coefficient ($r > 0.9$) and type 1 had greater slope than type 2 ($P < 0.01$). In another

study, 8 patients were studied using both the spontaneous or sequence method and the drug method. The two methods yielded BRS slopes that were highly correlated ($r = .96$, $P < .001$) (Parlow et al. 1995). Davies et al (Davies et al. 1999) compared four methods of BRS assessment: the sequence method, spectral analysis or the alpha index, controlled breathing time domain analysis, and the phenylephrine method, in 31 cardiac patients and 18 normal controls. In this study, all four measurements were done at the same time in the afternoon and two test episodes were obtained for each method of assessing BRS. The agreement among the four methods was tested with Bland-Altman plot. The most reproducible method was the controlled breathing protocol with a coefficient of variation of 19.6%. However, in the sequence method and the spectral analysis LF and HF, the CoV were 40.4%, 33.7% and 52.1% respectively. The phenylephrine technique was the least reproducible method with a CoV of 52.2%. More recently, Lipman et al who studied 97 subjects aged 25 to 86 years, using spontaneous BRS measurement and a modified phenylephrine measurement. They found that spontaneous indices after valsalva manoeuvre correlated poorly with the drug method and they were not useful as surrogates for baroreflex gain (Lipman, Salisbury, & Taylor 2003).

Mid- and long-term reproducibility of spontaneous baroreflex sensitivity was assessed by Herpin and Ragot ((Herpin & Ragot 1997). They studied 14 normotensive subjects using three noninvasive plethysmographic recordings of BP (Finapres). Two recordings were performed one week apart and the third one was performed one year later. BRS was estimated using the sequence method and the spectral analysis. The data were analysed using the Bland-Altman method; they did not find a significant difference between estimates of BRS from the three recordings

and the reproducibility of BRS measures were satisfactory. Measurements performed in the standing position had better repeatability than measurements made from supine subjects. The mid-term (1 week) coefficient of repeatability (CR) was 10.4 for sequence method and 3.8 for spectral analysis in the supine position, whereas for standing position the CR was 1.9 and 1.6 for sequence and spectral analysis respectively. Furthermore, the long-term (1 year) CR for supine position was 12.0 for sequence analysis and 4.7 for spectral methods. However, standing position was also more reproducible with CR of 2.0 and 2.2 for sequence and spectral analysis respectively. In another study (Iellamo et al. 1996) spontaneous BRS evaluation was moderately reproducible in two different days, 24 hours apart. Nevertheless, in this study, the CoV (coefficient of variation) was ranged between 13.9 and 19.7%. Dawson et al studied 39 normal subjects on two different occasions, whose breathing rate was controlled at 15 breath/minute. They found that the coefficient of variation ranged between 18.9 and 26.1%, but measurements obtained with the valsalva manoeuvre had a CoV of 16.8% (Dawson et al. 1997). More recently, an important study by Eckberg et al, showed that baroreflex sensitivity at low frequency varied from day to day in 9 healthy subjects. These baroreflex fluctuations were independent of the method used to estimate baroreflex sensitivity (Eckberg & Kuusela 2005). In most of the previous studies, researchers used some provocative mechanisms such as head tilting or valsalva manoeuvre in order to assess the reproducibility and validity of the non invasive measurement of baroreceptor sensitivity to give more power to the test. Eckberg studied the unprovoked fluctuations. The following table summarizes some of the studies

Author	No. of patients	Methods	Comments and conclusion
Parlow J (Parlow et al. 1995)	8 healthy subjects	Comparison between drug method and spontaneous method	The spontaneous baroreflex method provides a reliable, noninvasive assessment of human vagal cardiac baroreflex sensitivity within its physiological operating range
Iellamo F(Iellamo et al. 1996)	20 healthy subjects	Evaluation of reproducibility of spontaneous baroreflex sensitivity at rest and during laboratory tests	Spontaneous baroreflex method provides good BRS reproducibility under various stimuli that affect the neural control of circulation differently.
Herpin D(Herpin & Ragot 1997)	14 subjects three intervals 1st occasion after 1 week and the third one after one year	Mid- and long-term reproducibility of noninvasive measurements of spontaneous arterial baroreflex sensitivity in healthy volunteers	Noninvasive measures of BRS in standing position are more reproducible than supine position. The long-term reliability of the sequence method is higher than cross-spectral methods
Dawson S(Dawson et al. 1997)	39 subjects two occasions between 1 week and 6 months apart	The reproducibility of cardiac baroreceptor activity assessed non-invasively by spectral and sequence techniques	Noninvasive BRS techniques are reproducible and appear to be suitable for longitudinal studies of changes in cardiac baroreflex.
Davies (Davies et al. 1999)	A total of 31 patients with CHF and 18 normal controls Each subject underwent two test episodes with	Reproducibility of baroreflex sensitivity in normal controls and in patients with chronic heart failure.: (1) bolus	Of the four technique assessed for measuring BRS, the controlled breathing time-domain method yielded the best reproducibility and lowest failure rate in controls and in patients with chronic

	each method on the same day.	phenylephrine (BRS(Phe)), (2) alpha-index in both low- and high-frequency bands (BRS(alpha LF) and BRS(alpha HF) respectively), (3) the sequence method (BRS(Seq)), and (4) a new 0.1 Hz controlled-breathing, time-domain analysis method (BRS(Cbr))	heart failure.
Hojgaard M(Hojgaard et al. 2005)	14 healthy subjects In 3 different days	Reproducibility of heart rate variability, blood pressure variability and baroreceptor sensitivity during rest and head-up tilt	Spectral estimates of BRS had only moderate reproducibility (CV 14-20%).

Table 7.1 summary of BRS reproducibility studies

7.2 Factors that may affect BRS Reproducibility

Factors that may affect the autonomic neural outflow has already been considered (see Chapter 2). In this chapter, respiration will be considered as a major modulator of BRS.

Respiration

Breathing is a readily observed human rhythm that exerts profound influences on autonomic neural outflow. The most obvious manifestation of respiratory modulation of human autonomic rhythms is the respiratory sinus arrhythmia. Respiration gates the autonomic response to R-R interval fluctuations and it exerts influences on arterial pressure and muscle sympathetic nerve activity. Haymet & McClosky (Haymet & McCloskey 1975) found that vagal responses were greater when baroreceptor stimuli were applied in expiration than inspiration. In the same study, they found also that respiration gates the responsiveness of sympathetic motoneurones to changes in baroreceptor input. Another study suggested that respiratory gating of human vagal-cardiac responsiveness varied within the breathing cycle, that is, the human vagal response is most marked when baroreceptor stimuli begin in late inspiration and early expiration (Eckberg 1980). Gilbey (Gilbey et al. 1984) found that cardiac vagal motoneurones received an excitatory input during post-inspiration and powerful inhibitory synaptic input during inspiration. Badra (Badra et al. 2001) found that respiration greatly influenced the systolic blood pressure, R-R intervals and muscle sympathetic nerve activity. They also found low-frequency rhythms independent from respiratory activity as well as close correlations between arterial pressure, R-R intervals, and muscle sympathetic nerve activity at respiratory frequencies. These correlations resulted from the influence of respiration

on these measures rather than from arterial baroreflex physiology, which makes these autonomic and homodynamic rhythms vary over time and frequency. See Figure 7.1 below

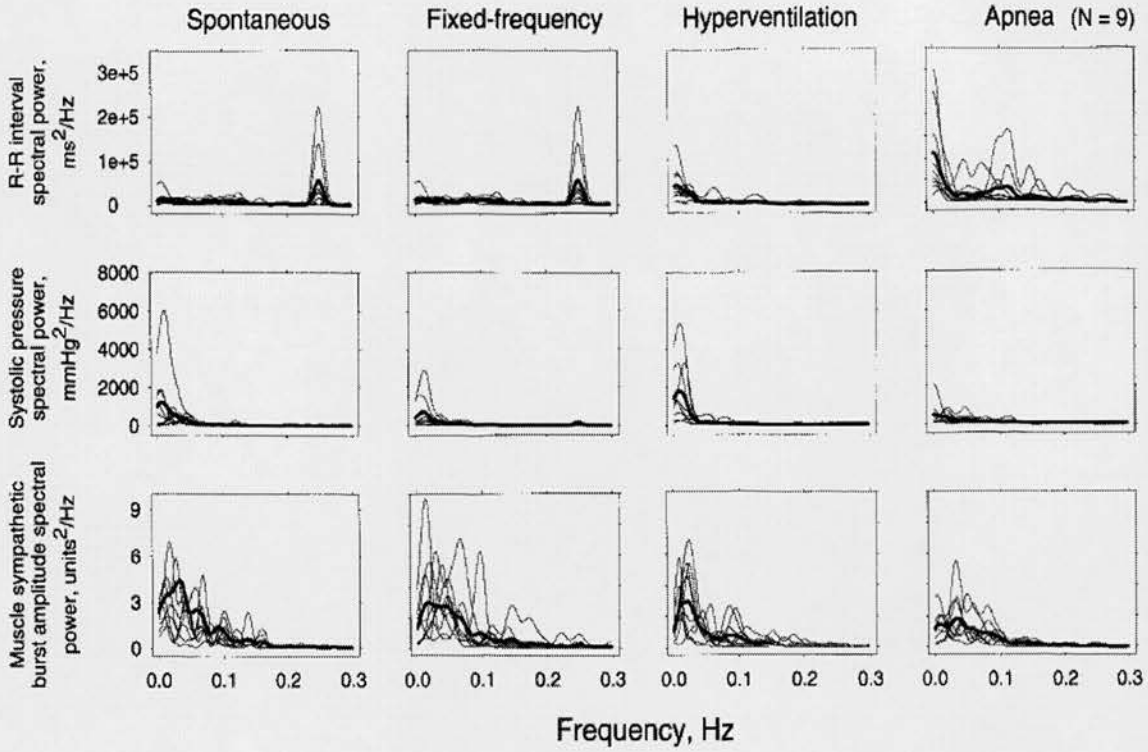


Figure 7.1 Power spectral densities from all subjects during different breathing patterns. light line in each panel are values from individual subjects, dark lines are group averages (Badra et al. 2001).

Recently, Rothlisberger et al, (Rothlisberger et al. 2003) found that baroreflex sequences occurred erratically at a frequency about one-third that of breathing. However, when baroreflex sequences did occur, the timing of their onset was dictated by the cycle of respiration. Parallel increases of systolic pressures and R-R intervals began just before and after the beginning of expiration, and parallel decreases of systolic pressures and R-R intervals began during late expiration and early inspiration. However, they concluded that these parallel systolic pressure and

R-R interval sequences were the expression of arterial baroreflex physiology, and the timing of such sequences within breaths reflected respiratory gating of muscle sympathetic bursts

The number of subjects used to study the reproducibility of spontaneous BRS measurement varied between 8 and 49. In our study, 7 healthy subjects were studied on three different occasions with no reproducibility as indicated by the coefficient of variation. In addition, 15 subjects attended for testing on two consecutive visits. These data were used to make a Bland-Altman plot.

7.3 Aim of the Study

The aim of this study was to test the reproducibility of BRS using the spontaneous method, with spontaneous breathing, since the initial placebo-controlled study has failed to show any significant difference between CPAP and placebo. A possible explanation for this finding could be that this method of measurement of BRS is not reproducible. To test this possibility, I used the spectral analysis of BRS to investigate the fluctuations in R-R intervals and systolic blood pressure over time. In addition, I tested the reproducibility of the spectral as well as the sequence method using CoV and Bland-Altman plot.

7.4 Subjects and Protocol

The study was performed in a similar way as explained above. Fifteen healthy subjects were recruited to perform the test. They breathed freely with no attempts made to control the frequency. Seven subjects completed three visits in three consecutive weeks and eight subjects were studied twice with one week in between.

7.5 Data Analysis

BRS was estimated as in the previous procedure, to obtain the sequence BRS and also the spectral analysis of BRS. We integrated power spectra of systolic pressure and R-R interval within frequency range, 0.02-0.15 Hz (VLF and LF frequencies), and took baroreflex sensitivity to be the square root of the ratio between the integrated spectra of the R-R interval and systolic pressure (total BRS).

BRS= $\sqrt{}$ (power R-R Interval/ power SBP)

We also used a more advanced programme to assess the data from some of the subjects.

The analysis was performed on data samples of five minutes, moving two seconds at a time, with analysis window width of 16 seconds. The same analysis was performed using three consecutive 5-minute intervals.

7.6 Data Shuffling

I studied the possibility that a random relationship between the systolic power and R-R interval power could cause similar values of BRS. To do this the input data were modified so that the relationship between the systolic blood pressure and the heart rate intervals was destroyed. This was done by interchanging the values for individual randomly chosen pairs of systolic blood pressure values. This resulted in the values for systolic blood pressure being "shuffled", but the R-R intervals and time values remained unchanged. The shuffling was done by exchanging SBP values from different positions in the time sequence. The diagram below explains this process. The entire sequence of the systolic BP values (> 1000) was taken and all the SBP values were shuffled which means that 1000 pairs of SBP and R-R intervals were repositioned. Values were chosen and exchanged with another values, both

chosen at random from the time series. In this way, the mean systolic blood pressure values in both series was kept the same.

Sequence number of time series	R-R Interval	Systolic pressure Unshuffled	Systolic pressure after shuffling
1	0.76	148.8	150.5
2	0.87	157.6	165.7
3	1.15	161.1	155.1
4	0.98	155.9	154.9
5	1.02	156.6	152.4
6	0.88	154.5	151.5
7	0.88	151.2	163.9
8	0.78	147.6	152.9
9	0.81	151.6	149.1
9	0.83	152.5	155.9
10	0.81	151.1	141.2
11	0.72	150.5	152.5
12	0.75	151.6	155.4
12	0.75	152.3	148.8
13	0.78	151.9	151.3
14	0.70	147.1	146.6
15	0.78	151.5	146.4
16	0.88	152.4	136.6
16	0.75	148.7	149.6

Figure 7.2 Diagram demonstrates the shuffling process.

The original data and the shuffled data were then subjected to Fourier analysis and used to calculate the BRS as before. Excel for windows was then used to analyse the reasons for variation in BRS.

7.7 Statistics

Reproducibility was tested using coefficient of variation for the subjects who attended the test three times (n=7). The whole sample (n=15) was plotted using a Bland-Altman plot.

7.7 Results

7.7.1 Characteristics of the Participants

Table 7.2 Characteristics of the Participants

	Mean	St. deviation
Age (Years)	32	7
BMI (kg/m2)	24.8	4.1
SBP (mmHg)	112	12
DBP (mmHg)	77	8

The participants were 9 males and 6 females who were not taking any medication and they were all non-smokers. They were young, not overweight, and normotensive

7.7.2 Preliminary Analysis

Figure 7.3 shows BRS from one subject in the first 5 minutes of recordings. The data indicate that BRS values fluctuate greatly over brief period of time with mean 15.4± 8.3 ms/mmHg.

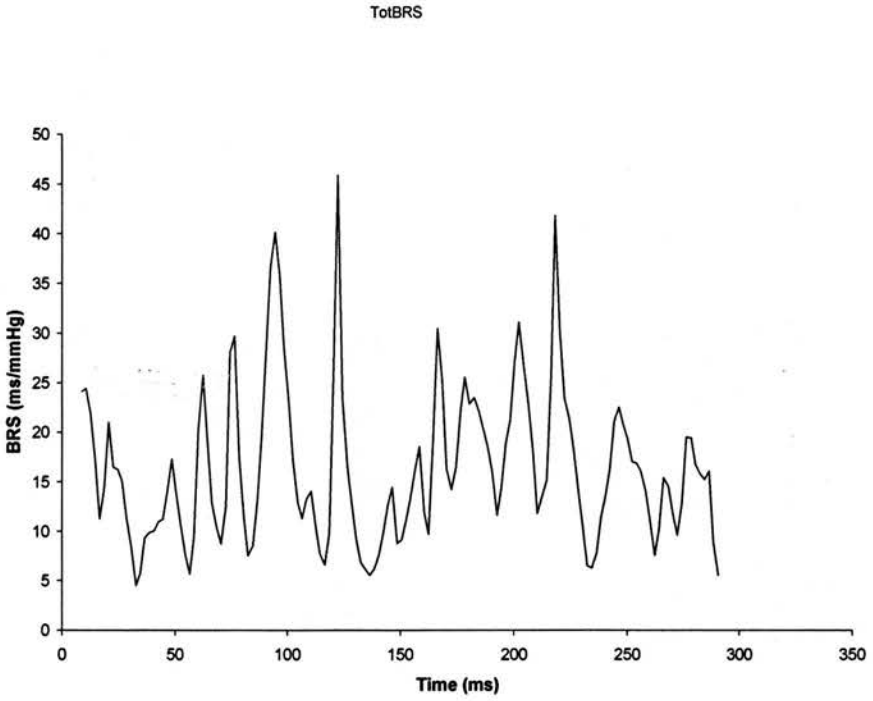


Figure 7.3 BRS fluctuation over 5 minutes period

We also studied one subject for 15 minutes continuously as it is shown in the figure 7.4, which indicates that BRS continue to fluctuate in spite longer period of analysis (window width). The mean was 14.7 ± 9.6 ms/mmHg

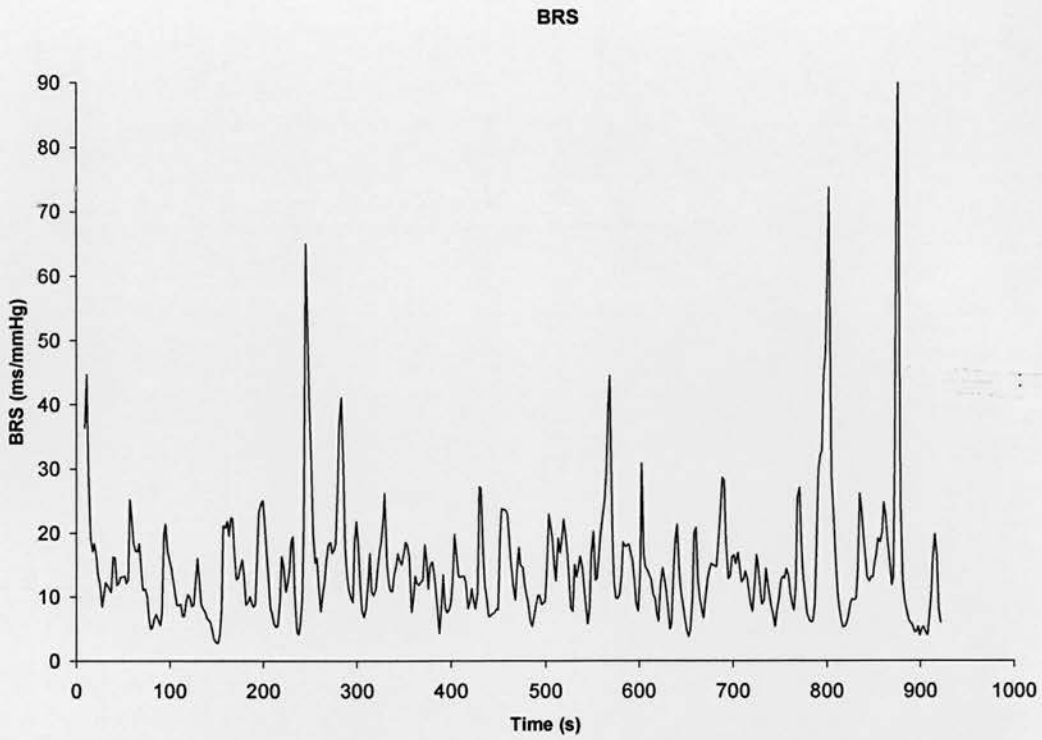
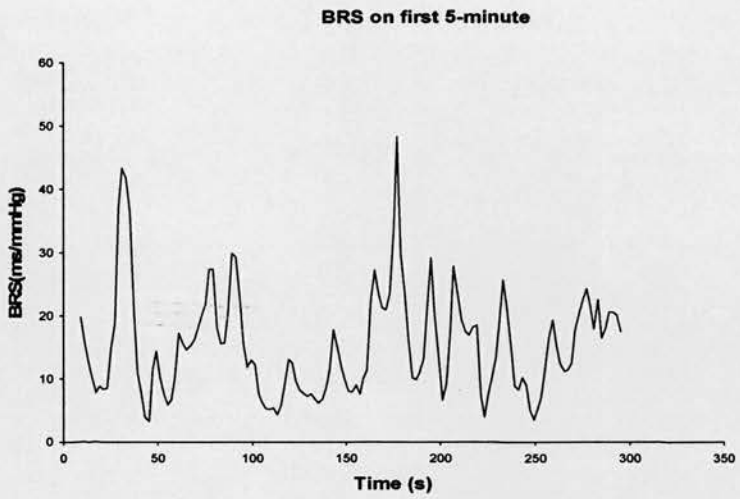


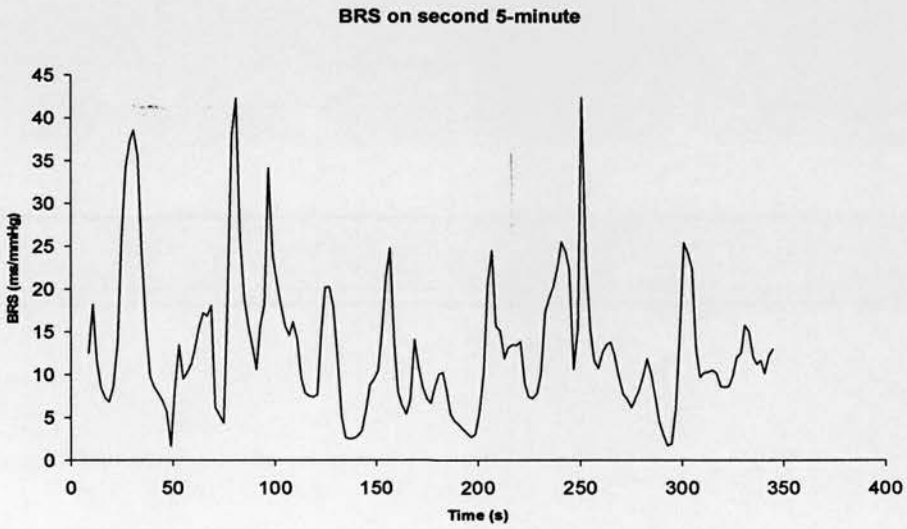
Figure 7.4 BRS fluctuation over 15 minutes period

Since we use to study our subjects in three 5-minutes intervals, I tried to demonstrate the variation of BRS in three consecutive 5-minute intervals which also was very marked. The mean of BRS in the first, second and third 5-minutes were also variable, 15.4± 8.3, 13±8, 13±9.6 ms/mmHg respectively

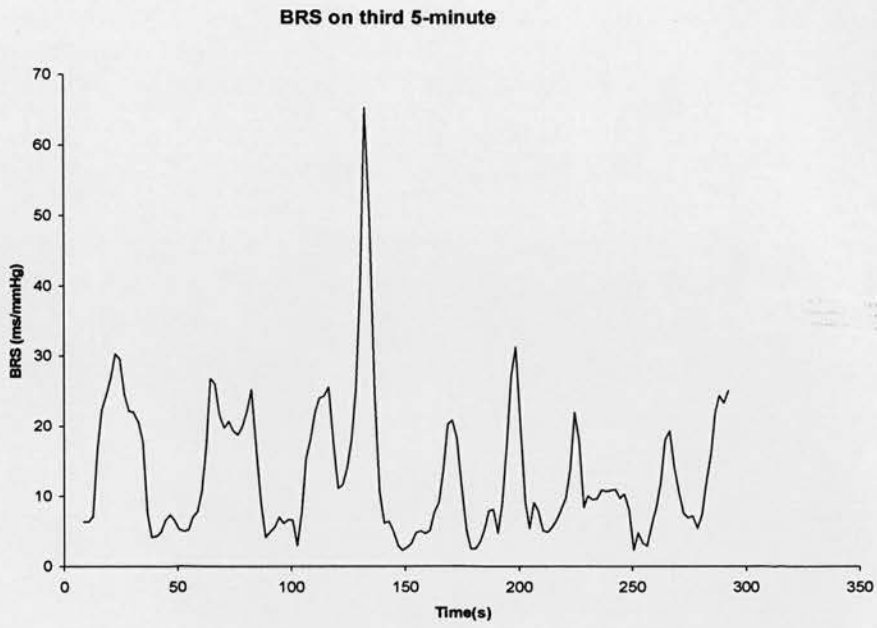
A


BRS on first 5-minute
BRS(ms/mmHg)
Time (s)
0
10
20
30
40
50
60
0
50
100
150
200
250
300
350

B


BRS on second 5-minute
BRS (ms/mmHg)
Time (s)
0
5
10
15
20
25
30
35
40
45
0
50
100
150
200
250
300
350
400

C



D

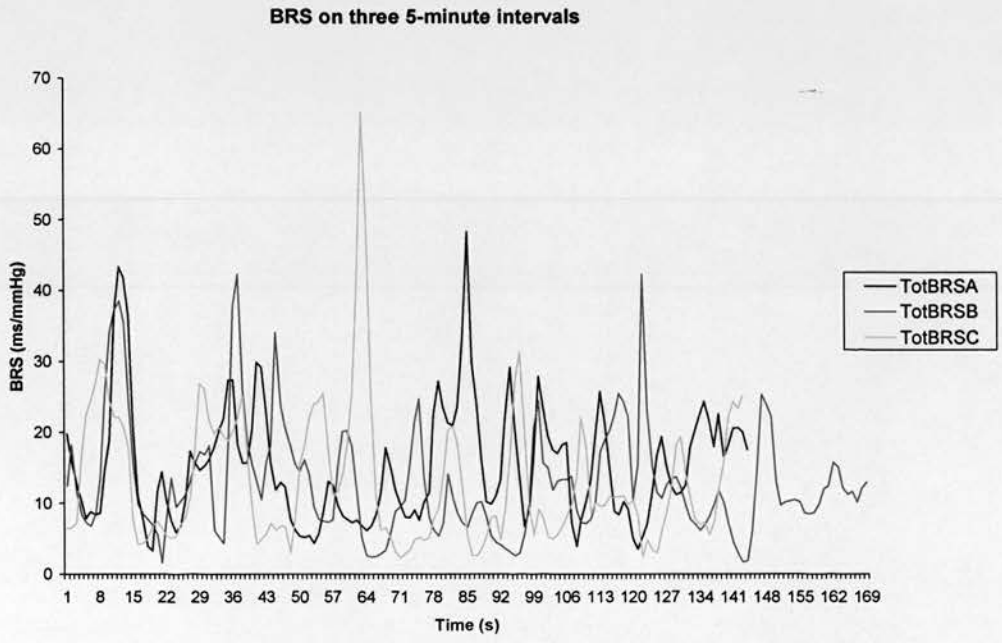
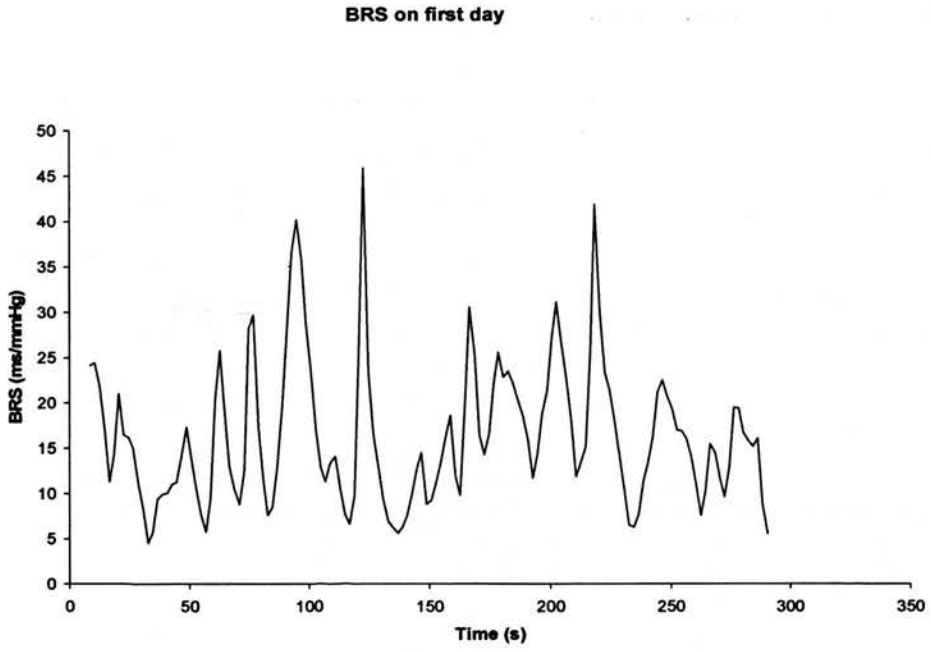


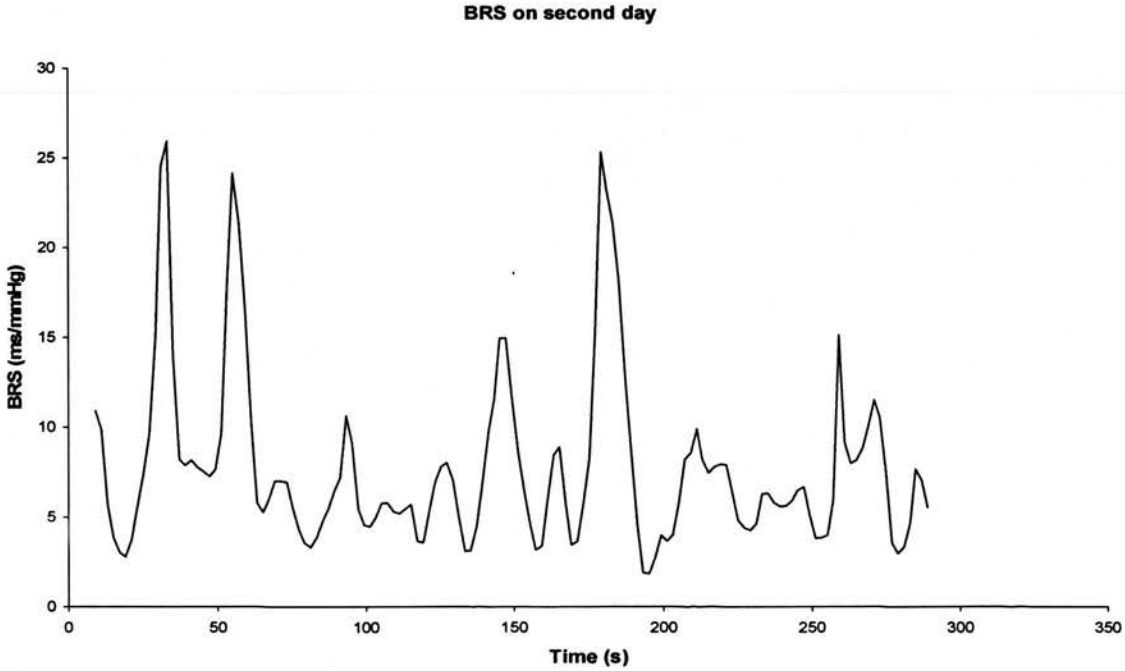
Figure 7.5 The BRS scores in three different 5-minutes intervals A, B &C. adding them together in graph D in which blue line indicates the first interval, whereas pink and yellow line indicates the second and third intervals respectively.

Similarly in figure 7.4, comparing the BRS results on three different days, shows that the variability of BRS is even more marked the mean of BRS for the first, second and third day respectively 16.3± 7.8, 7.8± 4.9, 12.1±8 ms/mmHg.

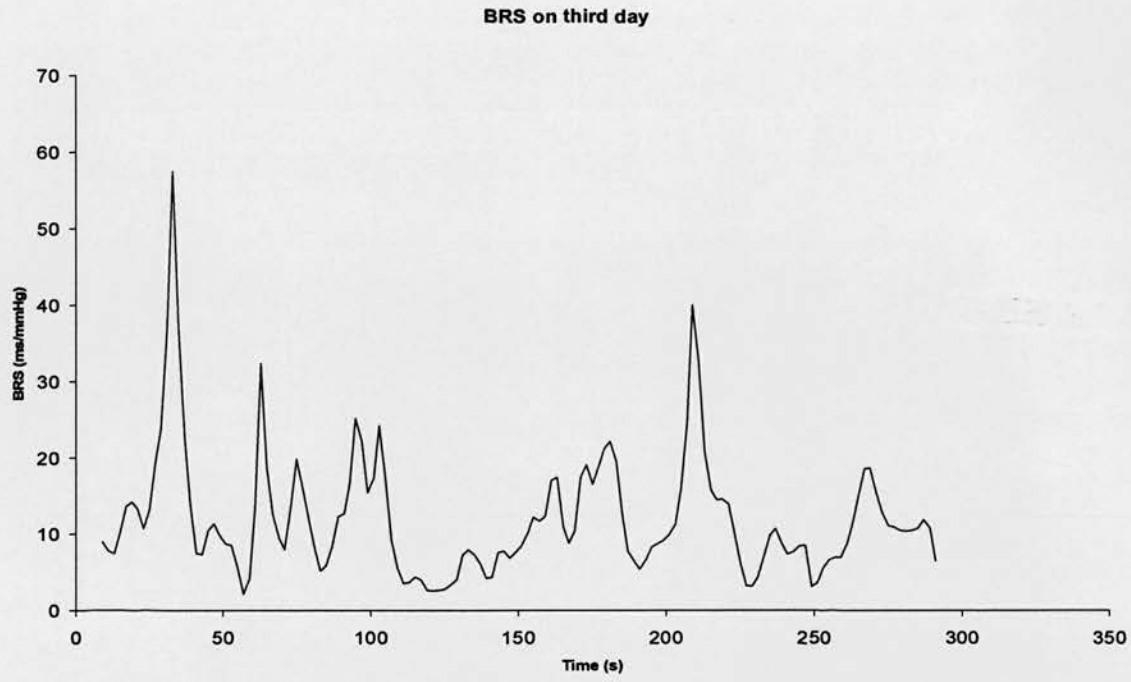
A


BRS on first day
BRS (ms/mmHg)
Time (s)
0
5
10
15
20
25
30
35
40
45
50
0
50
100
150
200
250
300
350

B


BRS on second day
BRS (ms/mmHg)
Time (s)
0
5
10
15
20
25
30
0
50
100
150
200
250
300
350

C



D

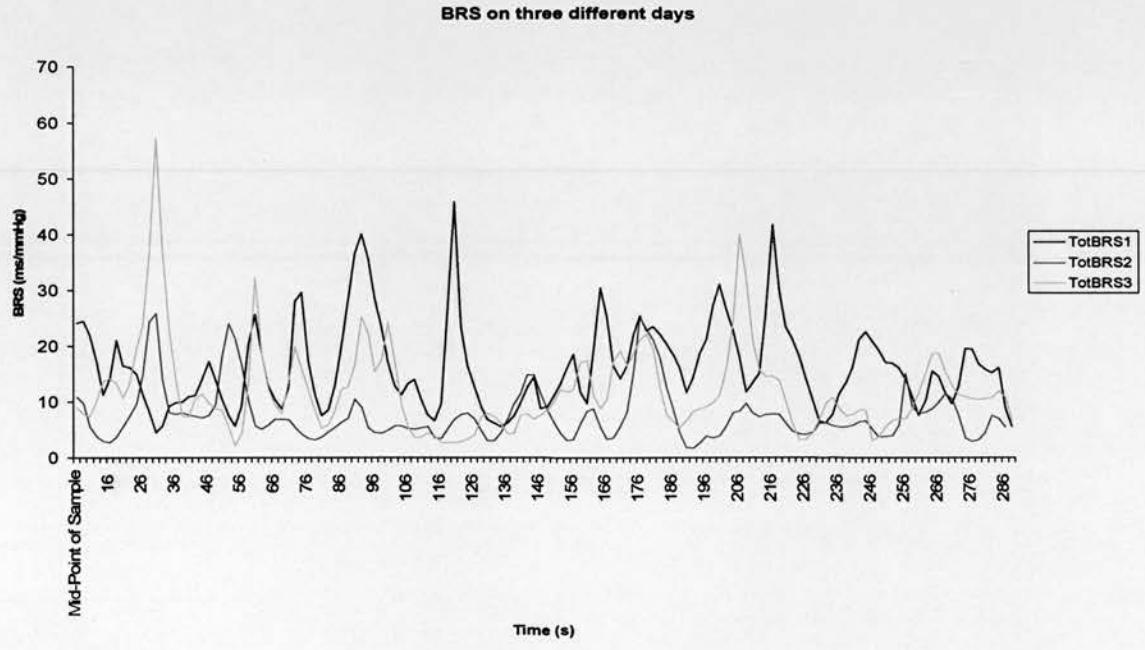
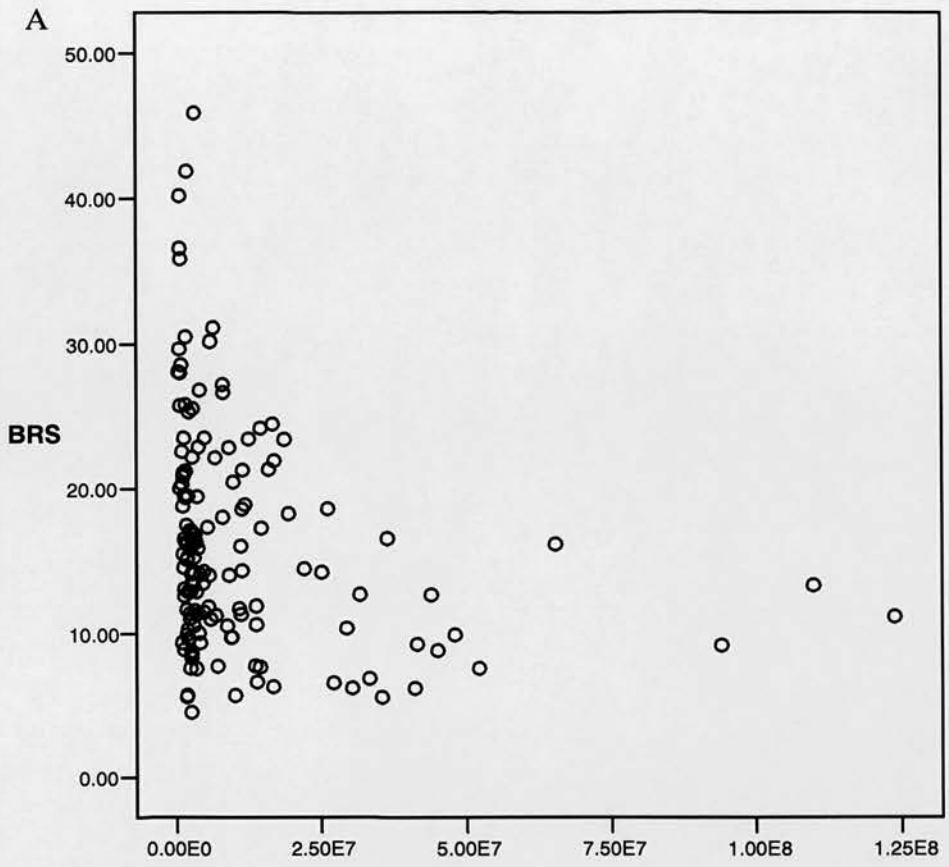


Figure 7.6 The BRS scores in three different days A, B & C. Graph D represent the three days together in which blue line indicates the first day, whereas pink and yellow line indicates the second and third day respectively.

7.7.3 Baroreflex Sensitivity

To study the cause of this variation in the BRS scores, data from one subject were examined in more detail. I considered the possibility that large values for the BRS could occur if the denominator in the formula became very small, that is if there was a negligible value for systolic pressure power. Total BRS scores were plotted against total spectral power of the systolic pressure, using power in very low and low frequencies and total spectral power of R-R intervals, figure 7.5. This plot shows that high BRS scores occurred when there was an association with a small value for the power spectrum of systolic pressure. The same relationship was inspected for the rest of the recording periods and similar results were obtained. In contrast, the plot of BRS in relation to the power of the RR interval did not show such an evident bias to the low power RR interval values


A
50.00
40.00
30.00
BRS
20.00
10.00
0.00
0.00E0
2.50E7
5.00E7
7.50E7
1.00E8
1.25E8
Total power of systolic pressure

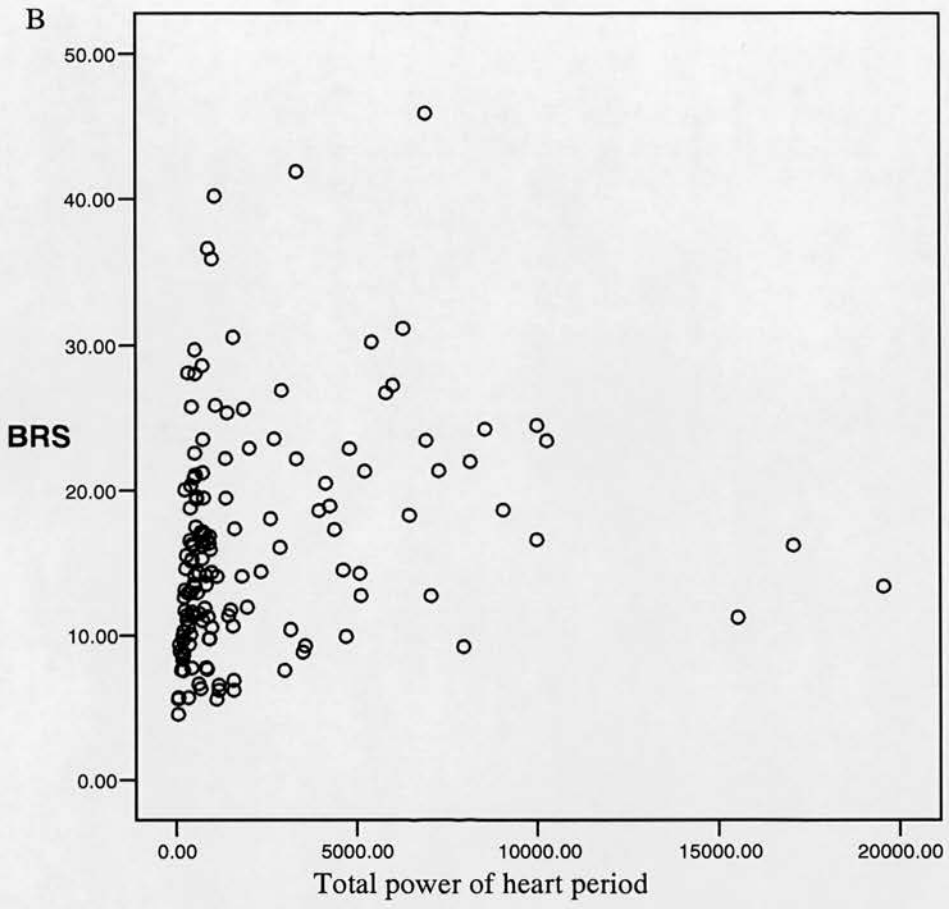


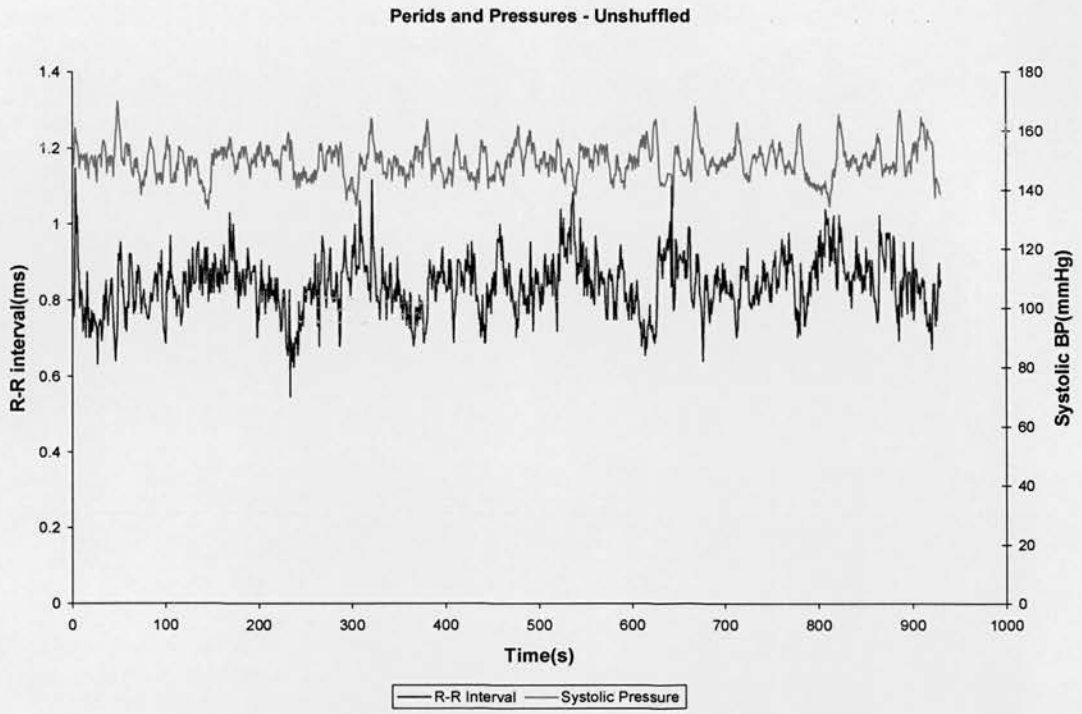
Figure 7.7 Total power of systolic pressure and heart rate A: indicates the correlation between total BRS and spectral power of systolic pressure; B: indicates the correlation between total BRS and spectral power of R-R intervals.

7.7.4 After Data Shuffling

We have studied one subject uninterrupted for 15 minutes (900 seconds, spectral width 2048 samples equal to 16 seconds of analysis time). We used data before and after shuffling of the systolic pressure values. Figure 7.7 suggests that shuffling the data and breaking the biological link between power of the arterial pressure and the R-R intervals does not make any difference in terms of reproducibility of baroreflex sensitivity. Table 7.3 below demonstrates the difference between shuffling and

unshuffling of the data. There was no significant difference (P=1.0) apart from total BRS scores and as expected total power of systolic pressure (P<0.0001). These results indicate that total systolic spectral power is the major factor in determining BRS fluctuation, which is also obvious from the graphs below, figures 7.6 and 7.7. These graphs indicate that there is major fluctuation in systolic pressure and with lesser extent with heart periods (R-R intervals) when plotting the row data of these two variables.

A



B

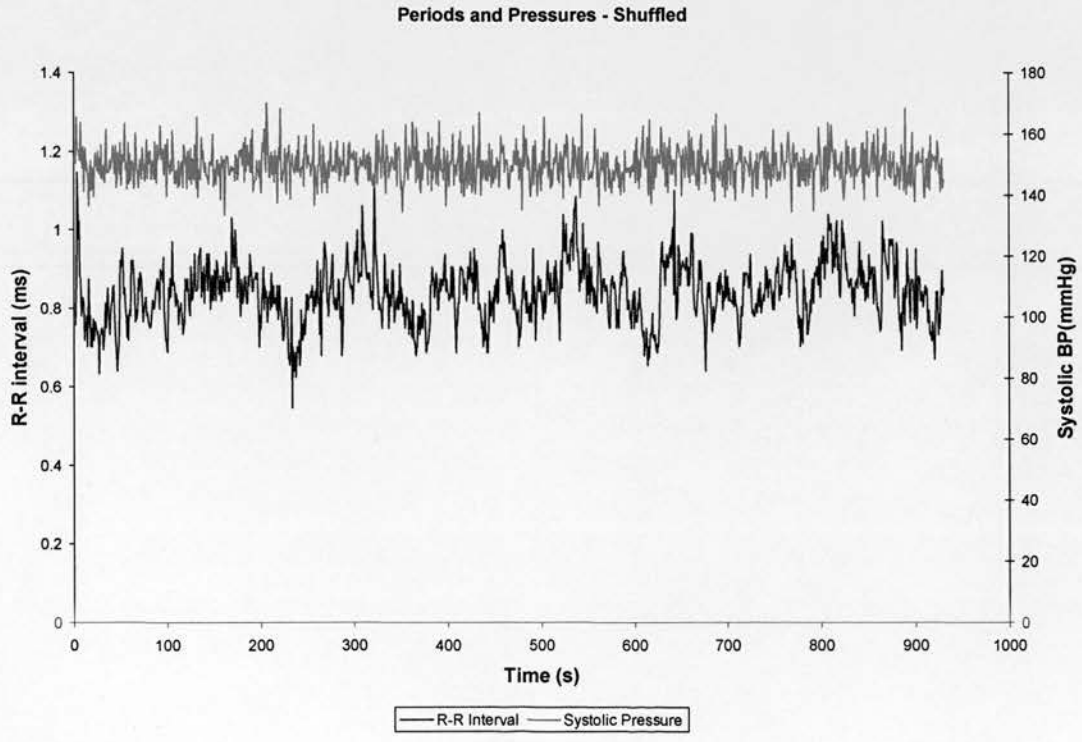
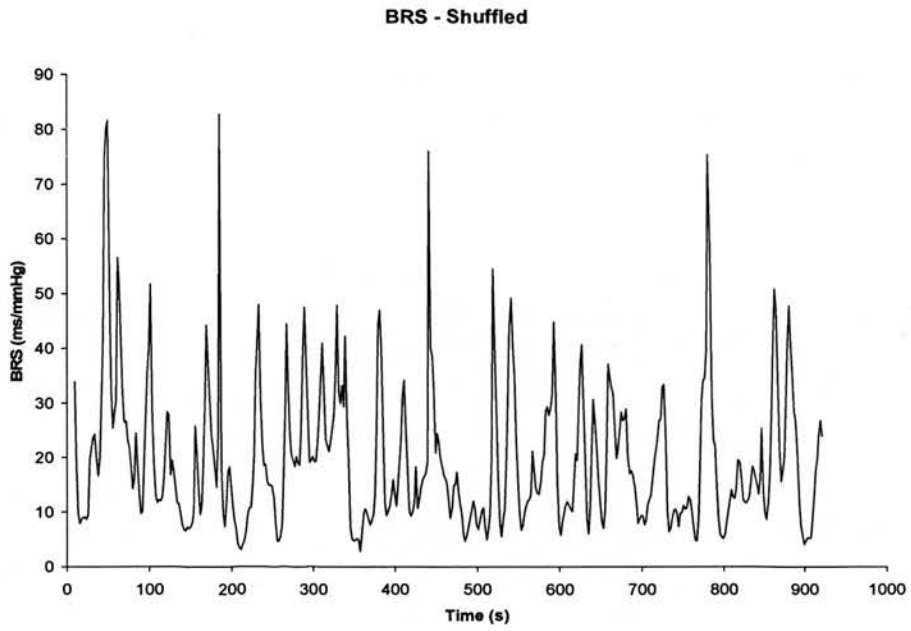


Figure 7.8 The two graphs show the row data the fluctuation of the systolic pressure and R-R intervals before (A) and after (B) shuffling.

A



B

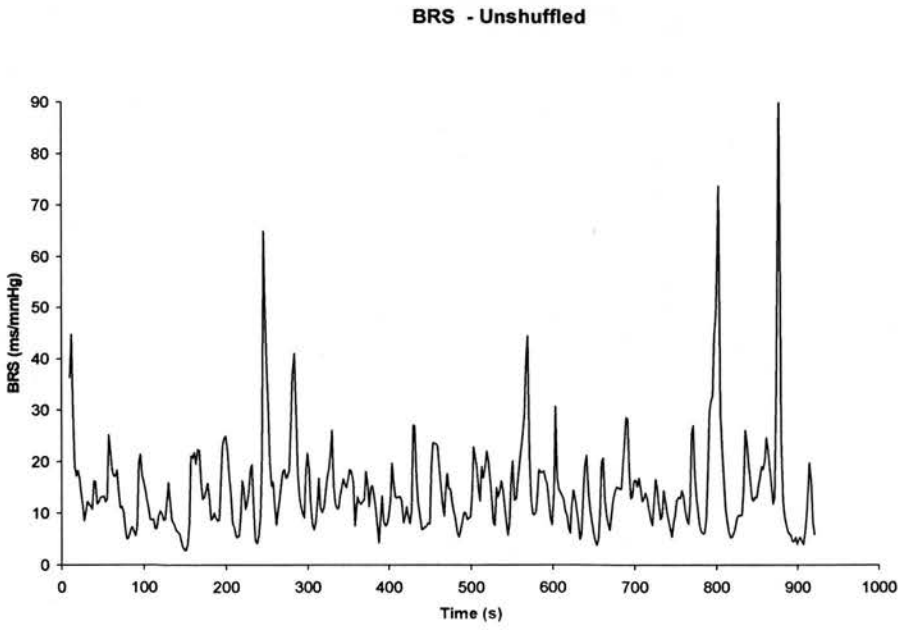


Figure 7.9 BRS before (A) and after (B) shuffling

Table 7.3 comparison between shuffled and unshuffled data

	Shuffled		Unshuffled		P
	Mean	SD.	Mean	SD	
Total BRS (ms/mmHg)	19.7	13.1	14.7	9.6	>0.0001
spectral period power (Hz)	1957.5	1900.6	1957.5	1900.6	--
Spectral systolic (Hz)	6880920	5503778	13974123	14627056	>0.0001
SBP(mmHg)	150	5	151	5	1.0
DBP(mmHg)	87	4	87.3	4.3	1.0
R-R intervals(ms)	0.8	0.08	0.8	0.08	1.0

7.7.5 Overall Analysis

Statistically the study was analysed in two ways; coefficient of variation (CoV) and Bland-Altman plot for reproducibility of measurements.

CoV (coefficient of variations)

Seven subjects attended for measurement of BRS test on three consecutive weeks at the same time of the day on each visit. The following tables show the BRS values of three visits. These values are BRS with sequence analysis and BRS with spectral analysis that include VLF, LF, HF and alpha.

Table 7.4 Sequence analysis BRS

BRS (ms/mmHg) (sequence analysis)	Visit 1	Visit 2	Visit3	COV %
Subject1	23.8	18.1	14.5	24
Subject2	40.7	22.3	39.6	30
Subject3	32.4	79.5	48.1	45
Subject4	37.4	47.2	56.7	18
Subject5	11.8	12.4	10.9	7
Subject6	13.9	18.0	13.4	17
Subject7	19.9	25.8	15.1	26
Mean				24 ± 11.9

Table 7.5 VLFBRS

VLFBRS (ms/mmHg)	Visit 1	Visit 2	Visit3	COV%
Subject1	14.7	9.2	13.8	23
Subject2	16.7	9.9	10.9	29
Subject3	8.2	12.7	15.8	31
Subject4	29.2	30.3	21.3	18
Subject5	5.4	5.6	7.2	16
Subject6	11.2	7.2	8.9	22
Subject7	10.1	20.0	5.6	26
Mean				23.6 ± 5.5

Table 7.6 LFBRS

LFBRS (ms/mmHg)	Visit 1	Visit 2	Visit3	COV%
Subject1	35.0	17.3	26.2	34
Subject2	58.7	19.1	44.0	49
Subject3	26.4	52.8	44.0	33
Subject4	81.0	60.1	68.1	15
Subject5	12.8	12.1	11.6	5
Subject6	24.9	19.0	20.6	14
Subject7	21.9	55.1	26.0	52
Mean				28.9 ± 18.1

Table 7.7 HFBRS

HFBRS (ms/mmHg)	Visit 1	Visit 2	Visit3	COV%
Subject1	117.3	45.2	68.5	49
Subject2	75.9	39.2	65.1	31
Subject3	77.1	49.3	71.6	22
Subject4	94.8	89.5	96.5	4
Subject5	28.6	21.8	19.5	2
Subject6	41.7	43.9	37.5	8
Subject7	28.7	92.4	66.8	51
Mean				23.9 ± 20.6

Table 7.8 Alpha BRS

Alpha BRS (ms/mmHg)	Visit 1	Visit 2	Visit3	COV%
Subject1	76.2	31.3	46.0	45
Subject2	67.3	29.2	53.0	39
Subject3	51.7	51.0	57.8	7
Subject4	87.9	75.2	82.3	8
Subject5	20.7	17.0	15.6	15
Subject6	33.3	31.4	29.2	7
Subject7	25.3	73.8	46.4	50
Mean				24.4 ± 19.4

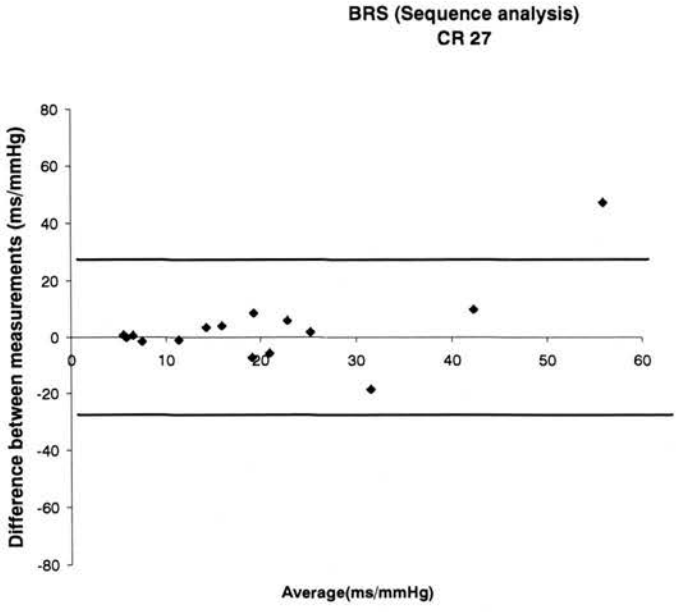
CoV: coefficient of variation. ([standard deviation / mean] x 100.)

The mean coefficient of variation for all the five variables was greater than 20 %, which indicates that the methodology of the study did not generate highly reproducible data. Tables 8.4-8.8 show that there is wide range of CoV between subjects. VLFBRS is less variable between subjects (16-26) compared to alpha BRS (7-50), HFBRS (2-51), LFBRS (5-52), and sequence BRS (7-45).

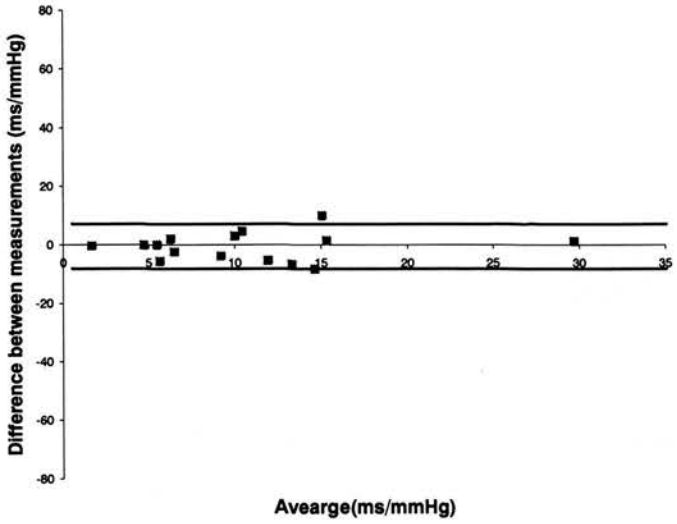
Bland-Altman plot

The Bland-Altman plot is a statistical method used to compare two measurement techniques. It may also be used to assess the repeatability of a method by comparing repeated measurements using one single method on a series of subjects (Bland & Altman 1986). Since for the repeated measurements the same method is used, the mean difference should be zero. The Coefficient of Repeatability (CR) can be calculated as 1.96 (or 2) times the standard deviations of the differences between the

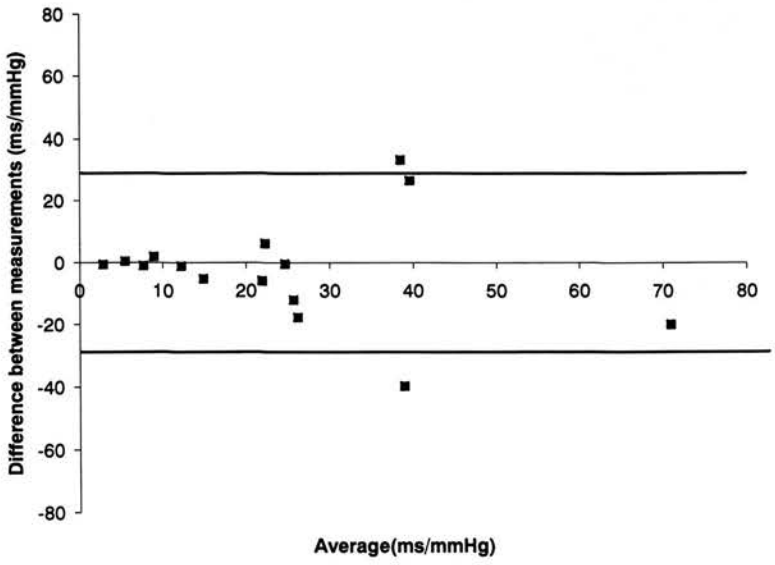
two measurements. In this study, I used Bland-Altman plots to test for the reproducibility of BRS measurement on two different occasions. In the figure 8.8 below I have plotted the difference between measurements against the average of the two visits for all the BRS measurement which includes sequential analysis, and spectral analysis, VLF, LF, HF and alpha BRS.



VLF
CR 8



LF
CR 28



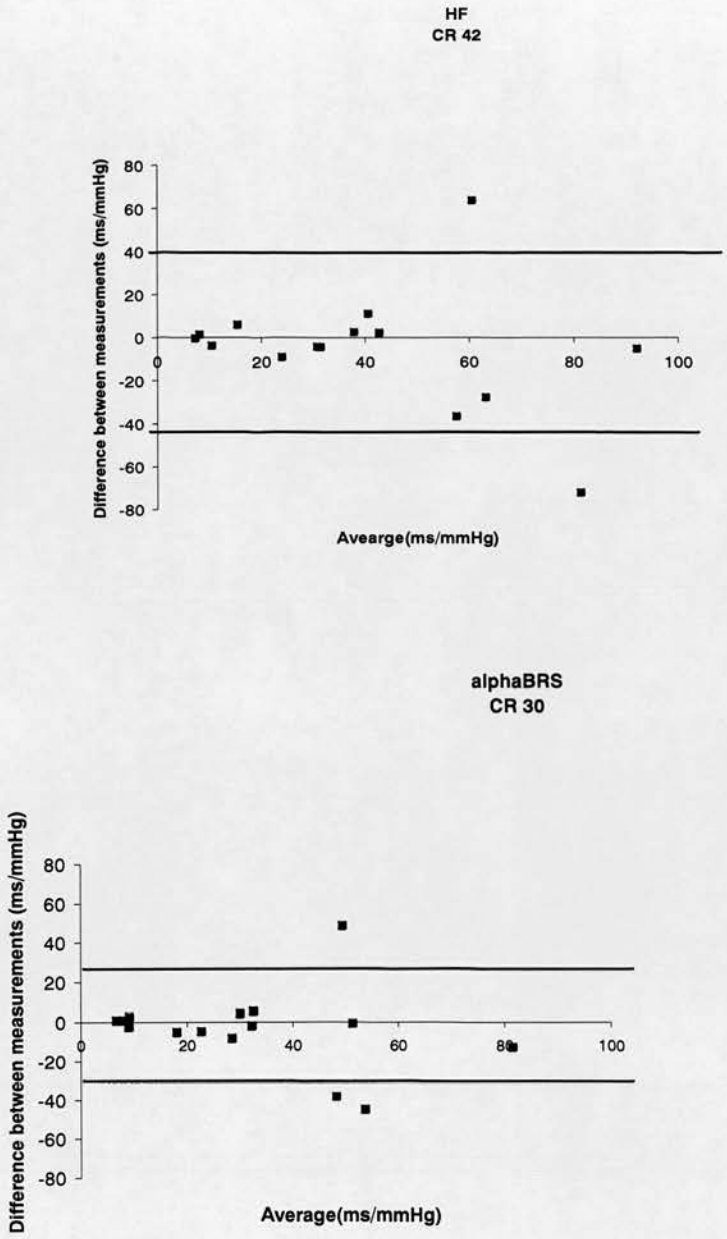


Figure 7.10 Bland-Altman plot for five parameters of spontaneous BRS measurements used in the study of BRS and OSAHS

These five graphs in figure 8.8 demonstrate the lack of agreement between the first and second visits. The coefficient or repeatability is large in BRS (sequence analysis)

(CR=27), LF (CR= 28), HF (42), alpha (30). VLF scores have the lowest coefficient of repeatability (CR=8)

7.8 Discussion

There are several methods of directly assesing baroreflex gain. As I have discussed above, these involve some perturbation that directly alters the system: the Valsalva manoeuvre, carotid occlusion, neck suction, vasodilators and/or vasoconstrictors, and postural changes. Recent research has made use of spontaneous oscillations in blood pressure and heart rate as we have done in this thesis. The baroreflex, as I have explained earlier in this thesis (Chapter 2), is the negative feedback that responds to arterial pressure changes by altering heart period, which may result in turn in arterial pressure changes. Physiologically it is difficult to assess baroreflex because it acts within a closed loop system. Thus, without opening the closed loop system, it remains uncertain whether heart period changes are responding to or adding to changes in pressure. Only by opening the loop can the output-to-input ratio of the feedback control system, termed gain or sensitivity be assessed. Nevertheless, the gain of the closed system can be assessed spontaneously without opening the loop, as in this study. It can be valid only if the input and output fluctuations of the system are sufficiently large, the system encompasses feedback relations alone, and if the gain is linear across all ranges. Unfortunately, all three of these conditions are difficult to meet in human baroreflex studies (Chen & Bishop 1983;Ludbrook 1984). Spectral estimates of BRS cannot explicitly distinguish between feedback and feed forward gain of baroreceptor function (deBoer, Karemaker, & Strackee 1987). Sequence analysis avoids this confound by limiting the data to only those fluctuations wherein pressure changes precede parallel heart period changes,

although only around 20% or fewer of beats in a time series fulfil these criteria (Bertinieri et al. 1988). Thus, the physiological assumptions upon which spectral and sequence indices rely are not the same, although they have been used interchangeably (Hughson et al. 1993)

This study investigated the reproducibility of baroreflex sensitivity provided by the spontaneous method in a group of healthy subjects who attended the test on more than one occasion. We have evaluated BRS by the sequence method and more importantly the spectral method. The analysis provides new insights into these measurement methods. Firstly, baroreflex sensitivity appears to vary considerably from minute to minute in subjects thought to be in a steady state. Secondly, this fluctuation of baroreflex values is also obvious from day-to-day measurements which could be oscillations occurring mainly at low frequency (Eckberg 1980;Eckberg & Kuusela 2005). Finally, the width of the window used for analysis does not have a major effect on baroreflex fluctuation, confirming the finding of Eckberg (Eckberg & Kuusela 2005)., nor on different spectral outcomes mainly VLF and LF.

7.8.1 Data Shuffling

To investigate further the cause of this variation I tried in this study to separate the relationship of R-R interval power changes from systolic blood pressure, by shuffling the SBP values. The aim of shuffling was intended to provide data that were not related; to see if the fluctuations were random or related to some link between R-R intervals and the systolic power signal. Shuffling showed that random variation could also generate large fluctuations in BRS. We have shown that systolic blood pressure is the major determinant in this fluctuation as it is shown from the tables and graphs above. However shuffling SBP values did affect the BRS values between shuffled

and unshuffled data. Thus could be because the coherent relationship between SBP and R-R intervals is disturbed.

This study compared the reproducibility of the spontaneous methods on more than one visit. We tried to show the reproducibility of the spectral analysis as well as the sequence analysis by two different statistical methods, coefficient of variation (Iellamo et al. 1996) and Bland-Altman plot (Davies et al. 1999), which had been used in other studies for the same purpose. Both statistical methods showed that repeat measurements of BRS are only weakly reproducible.

The reproducibility of these methods has been studied, before this study was started, as I have indicated in the introduction section. The reports of these studies did not indicate or emphasise the same lack of reproducibility as we have demonstrated here. However Lipman et al (Lipman, Salisbury, & Taylor 2003), did find that spontaneous methods are inconsistent with arterial baroreflex gain. In that study, baroreflex gain values fluctuated within a large range (12-27 ms/mmHg) making the limits of agreement for all indices greater than the estimated mean of the baroreflex gain of the population, thus indicating weak agreement. In the present study, the Bland ltman plots showed confidence intervals that often approached the magnitude of the measured variable, indicating a very limited confidence in these estimates. Furthermore, the CoV values obtained from measurements on three different occasions also suggest poor reproducibility of spontaneous BRS indices. Other studies have reported lack of reproducibility of spontaneous baroreflex indices. Maestri et al(Maestri et al. 1998), found only weak agreement between phenylephrine BRS and spectral indices in post-myocardial infarction patients. He attributed this variability to measurement errors in both methods. Similarly,

Colombo et al (Colombo et al. 1999), found weak agreement between the two methods in heart failure patients especially in uncontrolled breathing.

7.9 Conclusion

It is not likely that arterial baroreflexes are the sole reason for fluctuations of heart period paralleling those in systolic pressure. Spontaneous indices, either spectral or sequence analysis cannot be used to evaluate baroreflex gain since they cannot directly assess the actual changes that take place within the closed loop. Early studies of low frequency fluctuations in arterial pressure suggested that these could be caused by changes in central sympathetic activity (Preiss and Polosa 1974) Thus, these methods may be a poor way to evaluate treatment effects.

Chapter 8

Methods for the Study of Sleep Apnoea and Endothelial Function

8.1 Introduction

Vascular endothelium plays a key role in the biology of the arterial wall by the release of vasoactive factors. Clinical methods for the study of the endothelial function have recently developed considerably and endothelial function is now recognized as a marker for evaluating cardiovascular diseases as well as the benefits of drugs (Newby et al. 1999b;Newby et al. 1999a). In this section, the current methods available for testing endothelial function are discussed.

8.1.1 Circulating Biomarkers

Among current markers used in testing endothelial integrity, are Nitric Oxide metabolites, which are nitrites and nitrates metabolites were reported to be elevated in hypertension (Baylis & Vallance 1998). Nevertheless, these metabolites are strongly dependent on the diet. As discussed in Chapter 2, endothelin is also a biological marker for endothelial function. In contrast, high circulating levels of C-reactive protein (CRP) are well correlated with endothelial reactivity and are predictive of major cardiovascular events (Mulvihill et al. 2001). However, CRP level is influenced by non-vascular inflammatory phenomena, which must be taken into account for individual interpretation. Other inflammatory markers are also relevant. For example, levels of tumour necrosis factor-alpha (TNF-ά) were related to endothelium-dependent dilatation of skin resistance arteries in a heart transplant recipient (Holm et al. 2000). Furthermore, high levels of tissue plasminogen activator inhibitor are correlated with endothelial reactivity in hypertensive patients (Jansson,

Olofsson, & Nilsson 1993;Ridker et al. 1993). Recently, asymmetric dimethylarginine (ADMA) has been considered one of the biological markers for endothelial function and it is observed to play a role in hypertension and ischaemic heart diseases (Vallance et al. 1992b) (see Chapter2).

8.1.2 Brachial Artery Flow-mediated Dilatation

Post-ischaemic hyperaemia has been proposed as a method to evaluate the endothelial function of peripheral arteries. Blood vessels have the capacity to adjust blood flow in response to luminal physical and chemical stimuli (Joannides et al. 1995). An increase in blood flow will result in an increase in the shear stress to which the local vascular endothelium is subjected and the vessel responds by dilating, a phenomenon called flow-mediated vasodilatation (FMD) (Celermajer et al. 1992). High-resolution ultrasound imaging is used to measure the blood vessel, which is usually the brachial artery. A blood pressure cuff, placed either above or below the transducer position, is used to create the flow stimulus in the brachial artery. When placed above, the stimulus is greater, although accurate visualisation is more difficult. After baseline diameter measurement, the cuff is inflated to suprasystolic pressure for 5 minutes. Cuff deflation induces a brief high flow state (reactive hyperaemia) that subjects the endothelium to shear stress causing it to dilate (Alam, Seifalian, & Baker 2005). However, this procedure is operator-dependent and the ultrasonographic assessment of the brachial artery is challenging; therefore, the examination must be performed by well-trained sonographers.

8.1.3 Laser Doppler

Laser Doppler iontophoresis (LDI) is a non-invasive technique which was developed to avoid the inconvenience of venous occlusion plethsmography. Iontophoresis is

used to administer a minute quantity of a drug through the skin, using small electric currents. The underlying principle is that the molecules of a drug in solution are either positively or negatively charged, and will migrate across the skin under the influence of an applied monopolar current (Morris & Shore 1996). Acetycholine and sodium nitroprusside are used to generate endothelium-dependent and endothelium-independent vasodilatation, respectively. The procedure is carried out using very small currents (<100 μA) and it is painless. The forearm microvascular bed is usually the site of choice, and iontophoretic electrodes are attached to the volar aspect. The quantity of drug delivered is too small to have any systemic effects, although mild allergic reaction and skin irritation have been reported (Ramsay et al. 2002). Nevertheless, LDI technique is not without problems. The variability of skin conductivity in different populations must be considered when designing studies and interpreting results. A more significant problem is the tendency of the applied current to cause vasodilatation, even in the absence of an administered drug (Berliner 1997;Ramsay et al. 2002). However, LDI is becoming increasing popular since it is a non-invasive technique and provides a direct assessment of microvascular endothelial function.

8.1.4 Pulse-wave Analysis

Pulse-wave analysis (PWA) (Wilkinson, Cockcroft, & Webb 1998;Wilkinson & Webb 2001) is a fairly new technique that provides a non-invasive method of assessing global endothelial functions. Arterial stiffness is partly dependent on vasomotor tone, which in turn relies on an intact endothelium. As the arterial pulse waveform travels from the central circulation to the periphery, its shape provides a measure of systemic arterial stiffness, thus changes in the shape of the waveform will

partly reflect endothelial function (Hayward et al. 2002). PWA is new technology and it is also cheap, although it needs more validation with well-established methods such as venous occlusion plethsmography.

8.1.5 Venous Occlusion Plethysmography

This is the main topic of this chapter and it will be discussed in depth below.

8.2 Principle of the forearm study

The underlying principle of forearm venous occlusion plethysmography is that when venous drainage from the arm is interrupted for a short period, arterial inflow is unaltered, so blood can enter the forearm but cannot escape. This results in a linear increase in forearm volume over time, which is proportional to arterial blood inflow until venous pressure rises towards the occluding pressure. Most of the forearm blood flow is through skeletal muscle (70%) and most of the remainder circulates within the skin. The hand should be excluded during the measurement, for it has a different blood flow physiology and the blood flow might be non-linear (Wilkinson & Webb 2001).

8.3 Plethysmography

The procedure itself consists of inflating a cuff, placed around the upper arm, to above the venous pressure of around 40 mmHg. The cuff is inflated for 8 seconds, followed by deflation for 4 seconds. This manoeuvre does not alter the blood flow and allows venous emptying. The hands are excluded by a smaller cuff, placed around the wrist, inflated to above systolic pressure of around 190 mmHg. This cuff should be kept constantly inflated during the measurement of around three minutes. The forearm must be positioned above the level of the heart to ensure adequate venous emptying. Changes in forearm volume are measured by a plethysmography.

A strain gauge made of mercury-in-rubber is placed around the widest part of the forearm and acts as a resistor connected as one arm of a Wheatstone bridge. Changes in forearm volume result in a corresponding change in arm circumference and thus strain gauge length, which can be detected as an alteration in electrical resistance of the gauge, and thus potential difference. It is very important that the length of the gauge is equal to the resting circumference of the limb, and then changes in limb volume are directly proportional to the changes in resistance (Raitakari & Celermajer 2000;Webb 1995;WHITNEY 1949). The measure of blood flow to the forearm is usually expressed as ml per 100 ml of forearm volume per minute (Benjamin et al. 1994;Wilkinson & Webb 2001)

8.3.1 Drug Administration

venous occlusion plethysmography allows the study of the local effects of drug infusions (Webb 1995). Drugs can be infused intra-arterially by placement of a fine (27-gauge) needle into the brachial artery of the non-dominant arm under local anaesthesia, which may cause minimal discomfort. Minor problems such as bruising and local discomfort may appear at the time of cannulation, although they usually settle rapidly without any further problems. The needle is connected to an infusion pump by a tube with a similar calibre, which is set to deliver the appropriate flow rate. In addition, venous cannulation with 17-gauge cannula should be performed in both arms to allow blood sampling during the study.

8.3.2 Administered Drugs

Acetylcholine

Acetylcholine (Ach) is a potent endothelium-dependent vasodilator which acts on muscarinic receptors, primarily of the M3 subtype, to release NO from the

endothelial cells (Burning et al. 1994). It has a very rapid action with very short half-life (1 minute) and produces dilatation of almost all vascular beds, including those of the pulmonary and coronary vasculature. Although Ach is rarely given systemically, its cardiac actions are of importance because of the involvement of cholinergic vagal impulses. Small doses of Ach produce an evanescent fall in blood pressure because of generalized vasodilatation, accompanied usually by reflex tachycardia. If a large dose is given systemically, AV node block may be elicited with consequent bradycardia (Brown & Taylor 2001). In this study, Ach is infused locally with very small doses (5–20 micrograms) with great caution to avoid any systemic spillover. Blood pressure and pulse are measured regularly to check for any systemic effect as is indicated in the drug protocol below.

Substance P

Substance P is an endothelium-dependent vasodilator, which belongs to the tachykinin family of peptides. Substance P is present in the central nervous system, where it is a neurotransmitter, and in the gastrointestinal tract, where it may play a role as a transmitter in the enteric nervous system (Reid 2001). Substance P is also a potent vasodilator, producing marked hypotension in humans. The vasodilatation results from a direct action on arteriolar smooth muscle, which is mediated by the endothelial cell NK1 receptors. However, endogenous substance P does not appear to contribute to the maintenance of peripheral vascular tone or systemic blood pressure (Newby et al. 1999a).

Sodium Nitroprusside (SNP)

SNP is an endothelium-independent non-selective nitrovasodilator. It is metabolized by blood vessels to its active metabolite, nitric oxide. It dilates both arterioles and

venules, and the haemodynamic response to its systemic administration results from a combination of venous pooling and reduced arterial impedance. SNP is an unstable molecule that decomposes under strong alkaline conditions and when exposed to light, hence, it should be kept in a dark place or covered once it has been prepared. Its onset of action is within 30 seconds of infusion with the peak effect within 2 minutes. The effect disappears within 3 minutes of stopping the drug infusion. The short-term side effects of SNP are mainly limited to hypotension. This is unlikely to happen in this study, for very small doses (2–8 micrograms) are infused locally. Nevertheless, close monitoring of BP and pulse rate was regularly performed. Less commonly, toxicity may result from the conversion of SNP to cyanide and thiocyanate, which may lead to severe lactic acidosis. However, such toxicity might occur only if the drug is infused at a rate greater than 5 microgram/kg per minute (Oates & Brown 2001).

8.4 Protocol

8.4.1 Subject Recruitment

Consecutive new patients with obstructive sleep apnoea/hypopnoea syndrome confirmed by polysomnography at the Scottish National Sleep Centre, Edinburgh Royal Infirmary, were invited to participate in the study. The patients were divided in to two groups;

1. OSAHS with mild desaturations: AHI > 15, ESS > 11 with 4% desaturation index (4% DI) < 2.
2. OSAHS with severe desaturations: AHI > 15, ESS > 11 but the 4% DI > 20.

8.4.2 Exclusion

We excluded subjects who were unable to give written informed consent, subjects with respiratory comorbidity such as chronic obstructive airways disease, interstitial lung disease or pulmonary hypertension as defined on clinical and pathological grounds. We also excluded patients with significant kyphoscoliosis; daytime hypoxaemia of any cause defined as a oxygen saturation < 90% of breathing air; a partial pressure of carbon dioxide > 45mmHg, chronic heart failure; myocardial infarction within 3 months of the study; subjects aged < 18 or > 75 years; FEV1 < 65% predicted, subjects on ACE inhibitors and ACE receptor blockers; diabetic patients; those who had problems with sleepiness when driving; and shift workers.

All the plethysmography procedures were done in the Wellcome Trust Clinical Research Facility, Western General Hospital.

8.4.3 The Procedure

Written informed consent was obtained from each subject before entry into the study (patient information sheet appendix 4). The study was approved by the local ethics advisory committee. All participants had height and weight, and neck, waist and hip circumferences measured. The Epworth Sleepiness Scale was completed at the start of the study. Both groups had a full 48-hour ambulatory blood pressure recording before coming to the forearm study. Blood pressure was taken from the second set of 24-hour readings, allowing for adjustment to the cuff and device over the initial 24 hours (similar to the baroreflex study). For the forearm study, subjects were required to abstain from alcohol for 24 hours, and caffeine-containing drinks, tobacco, and food for 6 hours before each study. All studies were carried out at 0900 hours in a quiet, temperature-controlled room maintained at 22-24°C. Subjects lay recumbent

on a bed for the study duration. Semi-automated blood pressure cuff, venous occlusion cuffs, and strain gauges were applied. Pulses were checked. Intravenous cannulae and brachial artery needle were inserted under lignocaine local anaesthesia. A 27G needle was inserted in the non-dominant brachial artery. A 17G cannula was inserted into an antecubital vein in each arm for withdrawal of blood samples. Saline was infused at 1 ml/min to maintain patency of the arterial needle. Blood pressure and heart rate were recorded throughout. Forearm blood flow measurements were made every 20 minutes. After 30 min of saline infusion at 1 ml/min, intra-arterial substance P at 2, 4, 8 pmol/min, acetylcholine at 5, 10, 20 μg/min and sodium nitroprusside at 2, 4, 8 μg/min were infused for 6 min at each dose. There was a washout period of 20 minutes between each agent. Each patient had his or her drug order protocol randomly allocated by the research nurse to avoid any order bias. At the end of the study, cannulae were removed and haemostasis ensured. Pulses were rechecked. Subjects were observed for a period of 30 minutes and provided with a beverage before leaving the unit.

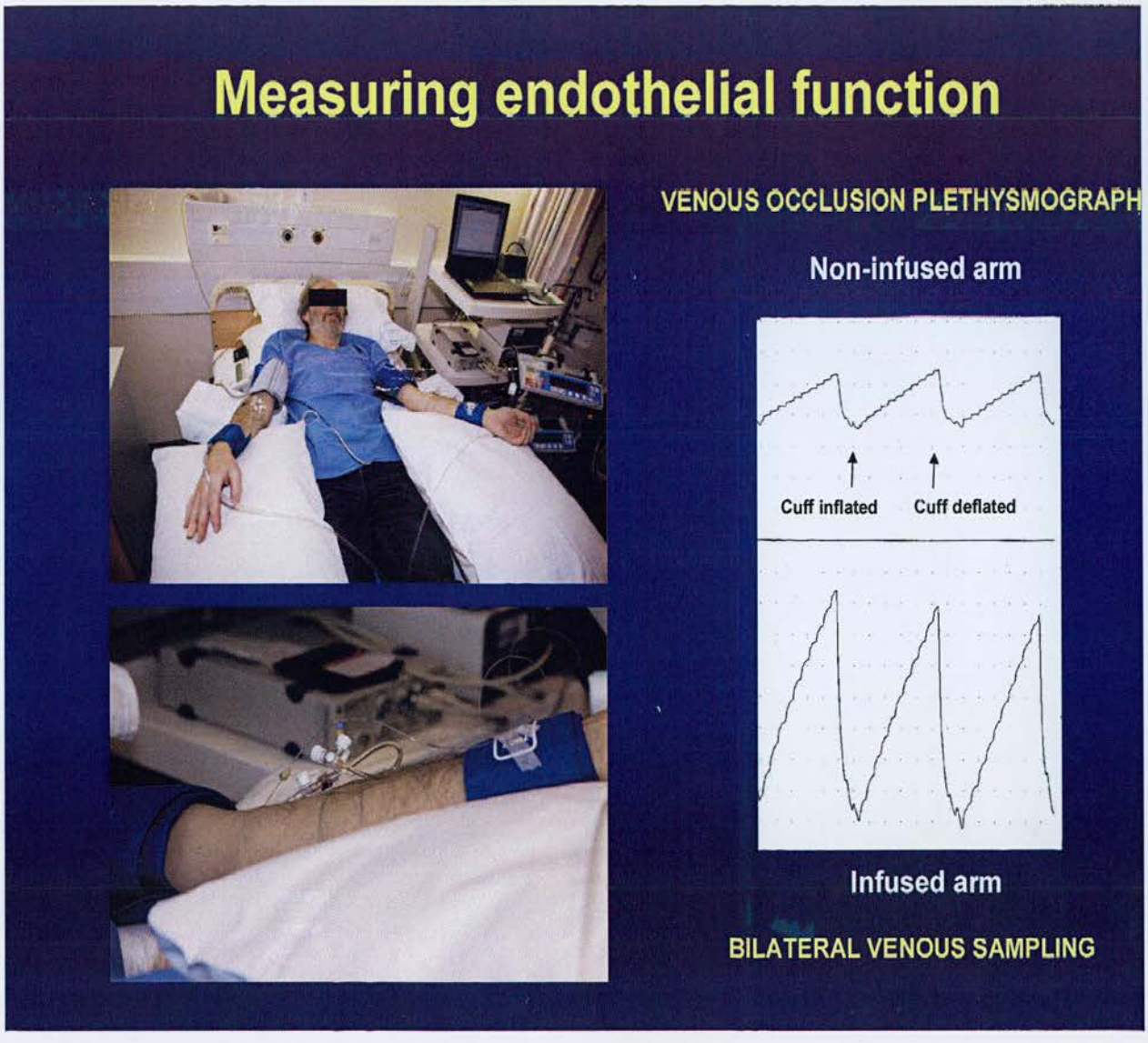


Figure 8.1 Measuring of endothelial function

Table 8.1 Example of drug order

Time	Vital signs	HR / min	BP / mm	Hg	Blood Flow	Infusion 1 ml/min	Blood sampling
-30 min						0.9% NaCl	
-27 min	√				√		
-17 min	√				√		
-7 min	√				√		√
0 min						Substance P 2 pmol/min	
3 min	√				√		√
6 min						4 pmol/min	
9 min	√				√		√
12 min						8 pmol/min	
15 min	√				√		√
18 min						0.9% NaCl	
25 min	√				√		
32 min	√				√		
38 min						Acetylcholine 5 µg/min	
41 min	√				√		
44 min						10 µg/min	
47 min	√				√		
50 min						20 µg/min	
53 min	√				√		
56 min						0.9% NaCl	
63 min	√				√		
70min	√				√		
76 min						Sodium NP 2 µg/min	
79 min	√				√		
82 min						4 µg/min	
85 min	√				√		
88 min						8 µg/min	
91 min	√				√		
94 min						0.9% NaCl	
101min	√				√		
108min	√				√		
114min						End	

Note that the order of agents varied with each patient

8.5 Data Analysis

Ten patients with a 4% oxygen Desaturation index (DI) ≥ 20 and ten patients with mild desaturation (DI 4% ≤ 2 & 3% DI≤ 5) were studied. Measurement of the forearm blood flow was done by chart for windows (chart4 for windows) programme. The scoring was calculated by taking the difference between the peak and the trough of the venous occlusion curve. The data were then moved to Excel for further processing.

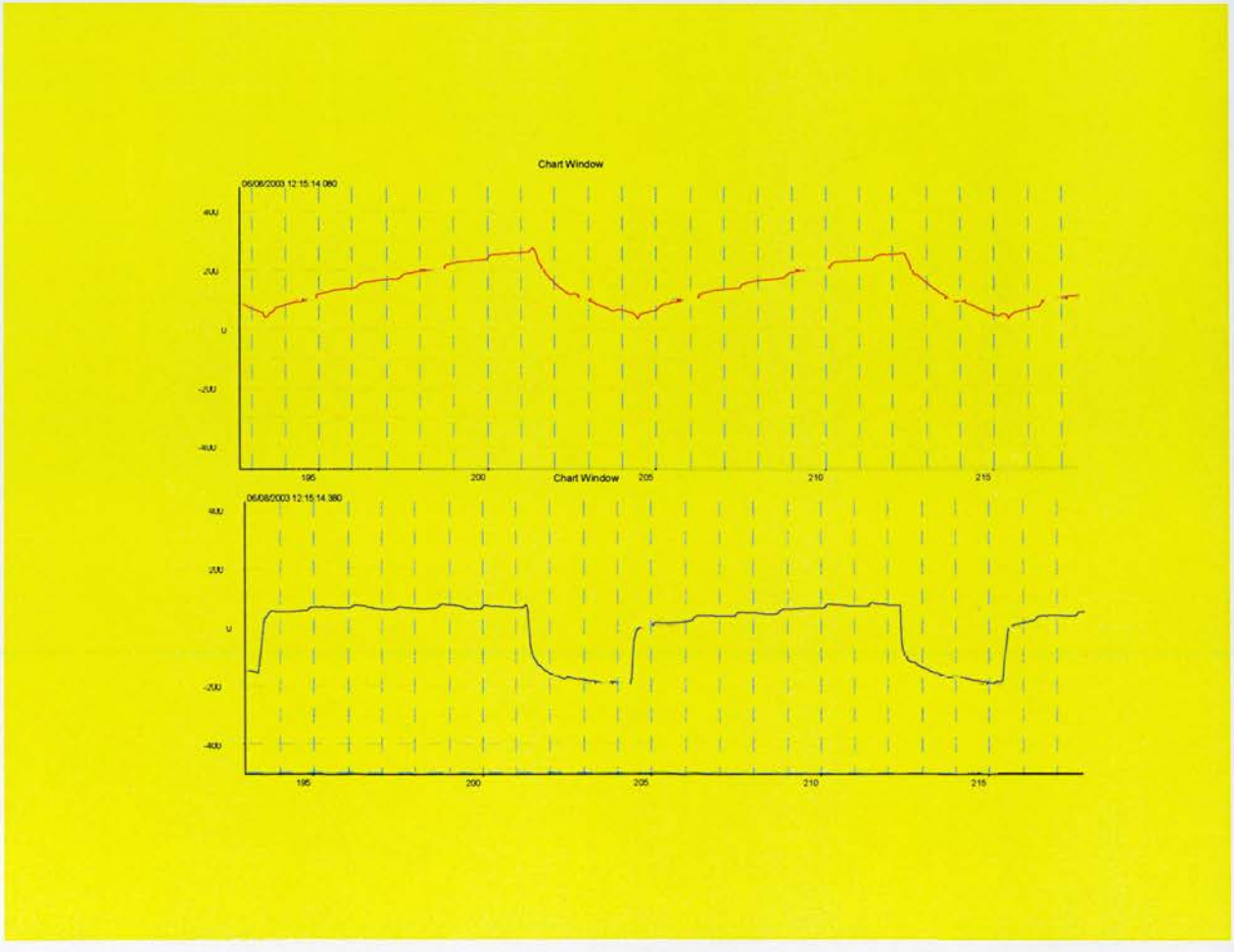


Figure 8.2 Example of plethysmography recordings: the upper section is the control arm and the lower section the infused arm.

Table 8.2 Example of the summary of changes of the forearm size that took place after infusion with each dose of the drugs used

	FBF:control arm	FBF: infused arm	% Δ:control arm	% Δ:infused arm	Inf/control arm	% Δ from predose
baseline1	1.11	4.00	-52.48	7.61	3.61	126.47
baseline2	2.33	3.72	0.00	0.00	1.59	0.00
SNP2	2.84	7.16	21.52	92.59	2.53	58.48
SNP4	2.25	9.02	-3.42	142.52	4.00	151.10
SNP8	2.99	11.41	28.30	206.90	3.81	139.21
washout1	2.85	5.75	-3.50	3.90	2.02	7.67
washout2	2.95	5.53	0.00	0.00	1.87	0.00
subp2	3.00	7.44	1.45	34.49	2.48	32.56
subp4	2.46	7.93	-16.65	43.39	3.22	72.03
subp8	3.08	10.63	4.18	92.13	3.46	84.42
washout3	3.39	5.47	11.06	20.66	1.61	8.64
washout4	3.06	4.54	0.00	0.00	1.48	0.00
Ach5	3.37	8.99	10.22	98.14	2.67	79.77
Ach10	3.50	8.03	14.47	77.00	2.29	54.63
Ach20	3.27	12.56	7.10	176.78	3.84	158.43

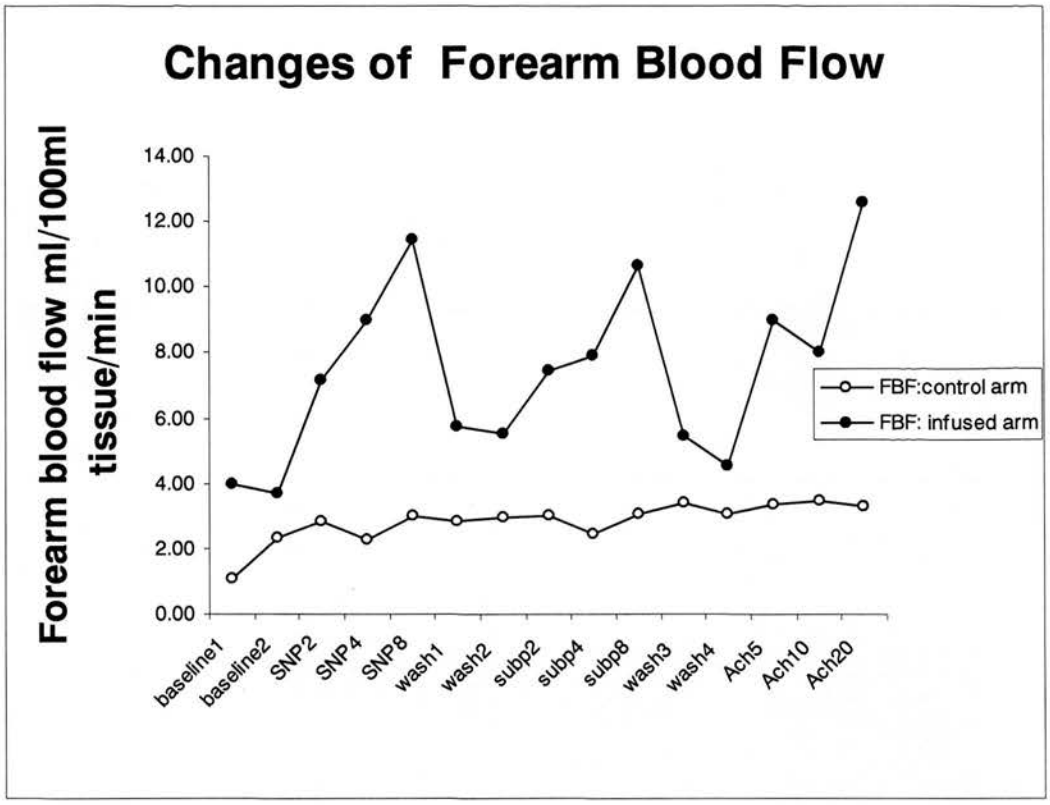


Figure 8.3 Changes in forearm blood flow in the infused arm compared to the control arm in one subject

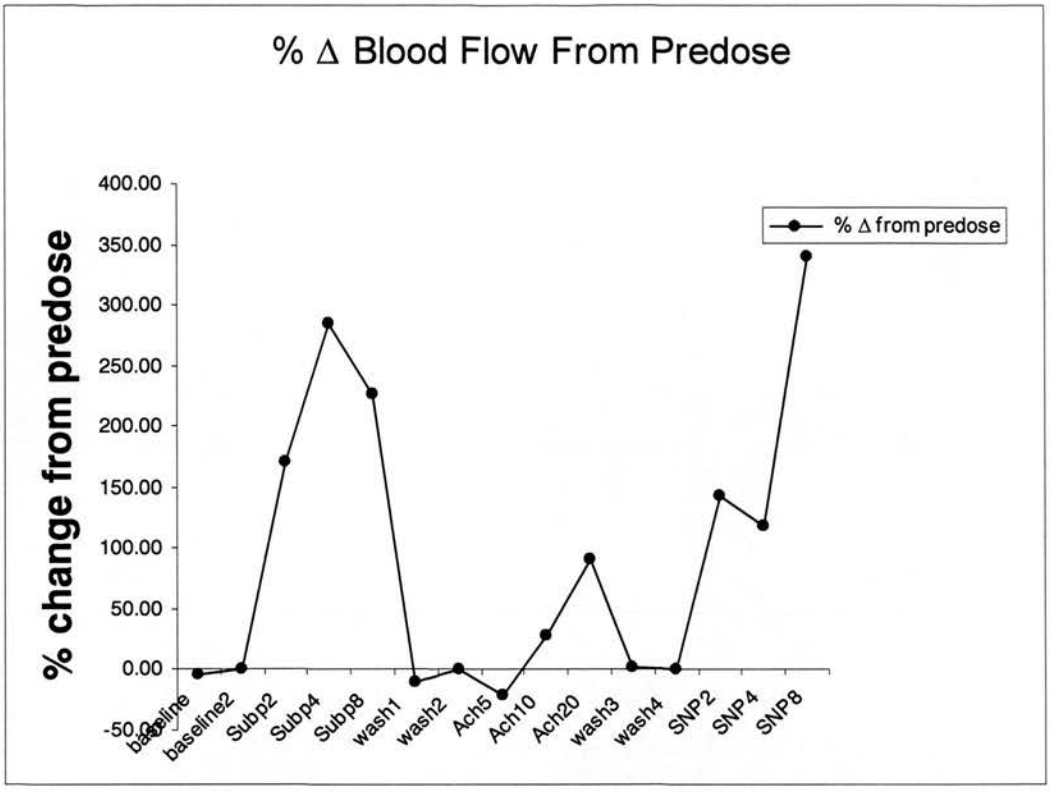


Figure 8.4 The percentage of change of forearm blood flow from predose in one subject.

Chapter 9
OSAHS and Endothelial Function Study Results

9.1 Introduction

This is an ongoing study of OSAHS and endothelial function. Twenty patients were included in the analysis: 10 male patients from the severe group (4% DI > 20), and 9 males and one female from the mild group (4% DI < 2 & 3%DI < 5).

9.2 Aim

The aim was to investigate whether hypoxaemia caused by OSAHS can impair the endothelial function. The hypothesis was that the severely hypoxaemic group might have an impaired response to vasodilating drugs, compared with the mild non-hypoxaemic patients.

9.2 Demographics of Patients

Table 9.1 compares the characteristics of the patients included in the analysis, using independent t-test.

Table 9.1 Comparison between mild and severe groups

	Severe group		Mild group		P-Value
	Mean	S.D.	Mean	S.D.	
Age	51	7	51	9	0.97
BMI	38.6	7.7	28.8	3.4	0.004
AHI	53.5	19.4	19	4.4	---
4% DI	42.9	15.2	1.5	0.9	---
ESS	17	3	14	3	0.08
SBP	136	12	129	10	0.2
DBP	80	11	80	8	0.9
Neck	45.5	3	42.7	1.5	0.04
Waist	122.3	17.3	98.3	10.2	0.01
Hip	123	16.9	103.3	4.8	0.02
Hip/waist	1.0	0.08	1.1	0.07	0.2

None of the patients in either group had ischaemic heart disease, hypertension or diabetes, although 2 of the severe OSAHS patients and 5 of the mild group were smokers. The patients in the severe group were more obese ($P < 0.05$, for BMI, and neck, waist, and hip size but not for hip/waist ratio P=0.2). Symptomatically, there was no statistical significance ($P = 0.08$), although there was a trend for the ESS for the severe group to be higher (17±3) compared with the mild group (14±3).

Nevertheless, there was no significant difference in the systolic or diastolic blood pressure.

9.3 Forearm Blood Flow Results

Based on the methodology of this study, the analysis of the data was performed in two stages, first for the control arm and then for the infused arm.

9.3.1 Statistics

The recordings of the plethysmography were scored using the Chart for Windows programme (see Chapter 9). The Independent-Samples T Test was used to compare the means for the two groups of cases.

9.3.2 Results

Table 9.2 Control (non-infused) forearm blood flow: comparison of mild and severe OSAHS

	Mild group		Severe group		P-value
	Mean	S.D.	Mean	S.D.	(2-tailed)
Baseline1	2.5	0.7	3.6	1.5	0.07
Baseline2	2.8	0.6	3.6	1.4	0.1
Pre Sub. P	2.6	0.6	3.7	1	0.02
Sub. P 2	3.1	0.4	3.7	1.3	0.1
Sub. P 4	3	0.7	3.7	1.4	0.2
Sub. P 8	2.9	0.8	3.8	1.4	0.09
Pre Ach	3.2	1	3.6	1.4	0.5
Ach 5	3.0	1	3.9	1.3	0.1
Ach 10	3.1	0.8	4	1.3	0.08
Ach 20	3.6	1.3	4.1	1.5	0.4
Pre SNP	2.8	0.9	3.9	1.4	0.07
SNP2	3.9	1.7	4.1	1.6	0.7
SNP4	3.3	1.2	4.1	1.4	0.2
SNP8	3.2	0.8	0.3	1.6	0.3

Values are expressed in ml/100 ml of forearm per minute.

Sub P = substance P Ach= Acetylcholine SNP= sodium nitroprusside

The data of the non-infused arms were normally distributed and an independent t-test was performed to obtain the p-value. Most of the results did not show any significant difference in absolute values of the perfusion between the two groups except pre-

substance P (P = 0.02). In addition, there was a trend with Ach10 μg and Substance P 8 μg (P = 0.08 & 0.09 respectively). Generally, the mean of the forearm blood flow study in the severe group was higher than in the mild group. The comparison of the severe and mild groups in infused arm is summarized in Table 9.4

Table 9.3 Comparison of the infused forearm blood flow in mild and severe OSAHS

	Mild group		Sever group		P-value
	Mean	S.D.	Mean	S.D.	(2-tailed)
Baseline1	3.1	0.8	4	1.6	0.1
Baseline2	3.1	0.9	3.6	1.3	0.3
Pre Sub. P	3.5	1.2	3.9	1.3	0.5
Sub. P 2	6.4	1.4	6.8	1.1	0.5
Sub. P 4	7	1.8	7.4	1.2	0.5
Sub. P 8	7.6	2.1	7.9	1.7	0.7
Pre Ach	3.6	1	3.6	1.2	1
Ach 5	6.6	2.8	5.8	2.2	0.5
Ach 10	6.6	2.7	6.6	2.4	1
Ach 20	7.2	2.4	7.4	2.8	0.9
Pre SNP	3.4	1.2	4	1.2	0.3
SNP2	7.5	2.3	7	1.2	0.6
SNP4	7.6	2	7.9	1.1	0.7
SNP8	8	2.2	9.1	1.6	0.2

Values are expressed in ml/100 ml of forearm per minute.

Using the absolute values of the study, there was no significant difference between the two groups in the infused arm at any stage (P > 0.1) with any of the three infused

drugs. During substance P infusion, the mean of forearm circumference was higher though statistically insignificant compared with the mild group. This finding has not been observed with acetylcholine and sodium nitroprusside drugs. The graphs in Figure 9.1 summarize the difference between the two groups, which also did not reveal any significant difference.

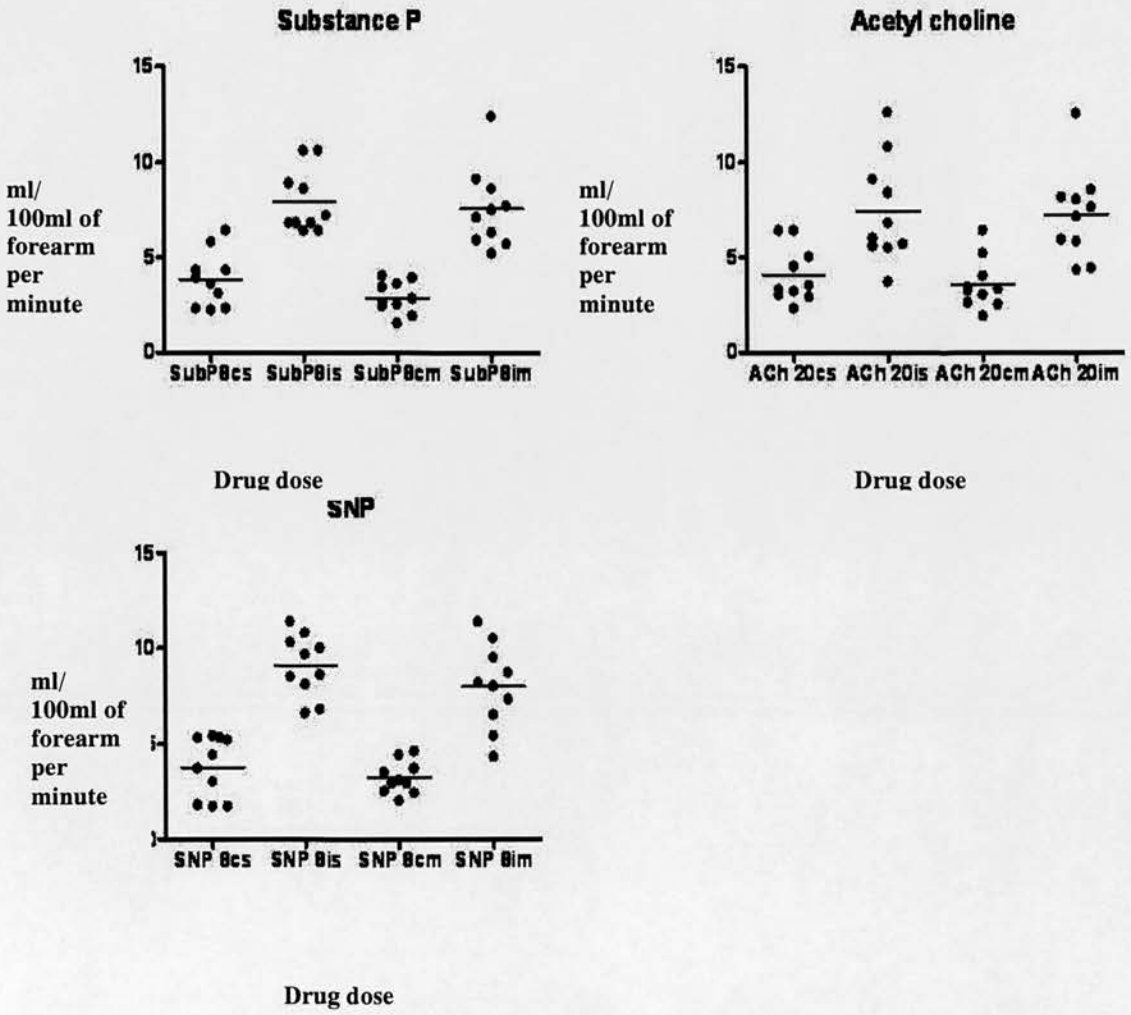


Figure 9.1 Difference between severe and mild group with maximum drug dose cs = control arm severe group; is = infused arm of severe group; cm = control arm mild group; im = infused arm of mild group

9.4 Conclusion

I tried in this study to assess the endothelial function in OSAHS patients in relation to the extent of nocturnal hypoxaemia. We used a comprehensive procedure to evaluate the response of vascular reactivity to hypoxaemia. This procedure is widely used to asses the endothelial function in cardiovascular diseases (Newby et al. 1999a;Webb 1995). To the best of my knowledge at the time this study was started, it was the first to assess endothelial function in OSAHS patients using forearm blood flow technique in relation to nocturnal hypoxaemia. My initial hypothesis was that severe hypoxaemic OSAHS patients would have impaired endothelial function compared to mild OSAHS patients. However, the null hypothesis could not be rejected concerning vascular reactivity between severe and mild OSAHS. This may reflect a genuine lack of difference, a lack of power in this study or other differences, since patients with severe OSAHS were more overweight compared with the mild group, which may result in technical difficulties in measuring forearm blood flow.

As discussed in chapter 2, impaired endothelium dependent function and endothelium independent function in the forearm vascular bed is associated with an increased risk of acute cardiovascular events, including cardiac death (Newby et al. 1999b;Newby et al. 2001). The endothelium is a major target of oxidative stress, and this stress may play a role in the pathophysiology of vascular disease. In OSAHS, recurrent episodes of hypoxaemia followed by re-oxygenation may trigger endothelial damage, via oxidative stress, superoxide radical formation (Row et al. 2003), its combination with nitric oxide and reducing nitric oxide bioavailability in the vessel wall, which leads to vasoconstriction. This study did not show that hypoxaemia has an effect on endothelial function, which could have been caused by

the above-mentioned factors. However, more recent studies have shown that oxidative stress and lipid preoxidation do not appear to be the key mediator for the cardiovascular diseases in OSAHS (Svatikova et al. 2004;Svatikova et al. 2005). This finding contradicts the results of other studies which showed that OSAHS patients have an increased status of oxidative stress such as thiobarbituric reactive substances and peroxides (Lavie 2003) and decreased antioxidant capacity which could be reversed by CPAP treatment (Barcelo et al. 2006;Lavie, Vishnevsky, & Lavie 2004). More randomised case-control studies with larger samples are required to clarify the role of oxidative stress in the development of cardiovascular diseases in sleep apnoea patients.

Further details will be discussed in the final chapter.

Chapter 10

Hypoxaemia and Hypertension in Patients with Obstructive Sleep Apnoea Hypopnoea Syndrome

10.1 Background

As discussed earlier, hypoxaemia could trigger an increase in sympathetic activity, mainly through chemoreflexes, which could increase systemic blood pressure (Narkiewicz et al. 1998c). The triggering of chemoreflexes to hypoxia is cannot be explained only by obesity, since obese subjects who are otherwise healthy with no OSAHS have chemoreflex responses similar to those seen in control subjects (Narkiewicz et al. 1999a;Narkiewicz et al. 1999c). In addition, both hypoxia and hypercapnia have local vascular effects, causing vasodilatation, which lowers the blood pressure initially, which in turn causes an increase in sympathetic stimulation and release of catecholamines (Shepard, Jr. 1990). Furthermore, hypoxaemia might perhaps cause endothelial dysfunction, which consequently causes hypertension (see Chapter 2)

10.2 Aim of the Study

The study aimed to determine putative predictors of hypertension in OSAHS patients.

10.3 Methods

Ambulatory blood pressure monitoring was measured as described in Chapter 3.

Hypertension was defined by:

1-patients taking antihypertensive treatment

2-patients found to be hypertensive on 24-hour ambulatory blood pressure monitoring with mean systolic and diastolic blood pressure more than 140/90 mmHg

Desaturation status was determined by 4% decrease of oxygen saturation from the baseline detected during sleep studies (see chapter 3)

10.4 Subjects

Thirty-nine patients participated in this study as part of the endothelial function study, and they attended the Department of Sleep Medicine at the Royal Infirmary of Edinburgh.

10.5 Results

10.5.1 Statistics

The data were sought by forward conditional logistic regression and tested by confirmatory analysis.

10.5.2 Patients' Demographics

Table 10.1 Patients' demographics

	Mean	SD.
Age (years)	50	9
AHI	45.8	28.7
ESS	16	3
BMI (kg/m2)	34.8	7.8

Nineteen patients of 39 patients had hypertension (14 by ABP and 5 by anti-hypertension treatment criteria), 24 were nocturnal desaturators and 32 non-dippers (< 10% nocturnal fall in mean arterial BP).

10.5.3 Forward Regression of Determinants

Forward regression of determinants found that increasing age (exp B 1.11 per yr, p = 0.03) and desaturation (exp B 0.15, p = 0.02) were significant independent predictors for hypertension, together explaining 31% of hypertension variance. Forcing known hypertension risks into a confirmatory model (age, BMI, male gender, AHI, and loss of nocturnal BP dipping) (Table 10.2 and Figure 10.1) explained 30% of variance (step 1), estimating a 12% increased odds of hypertension with each added year of age and 3% increased risk with each unit rise in AHI (p = 0.03). Those with male gender, BMI and loss of nocturnal BP dipping (all p >0.6) accounted for 30% in hypertension variance. In step 2 (forward conditional regression), desaturation explained an additional 11% of variance in hypertension (R^2 41%).

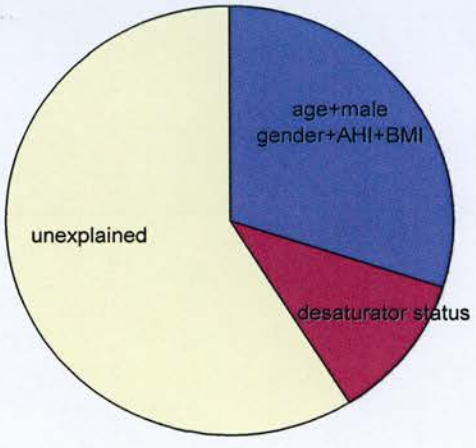


Figure10.1 The contribution of hypertension by different factors

Table 10.2 Summery of the analysis

Step	Variable	p-value	expB	Total Variance explained (R^2)
1 (forced)	Age (years)	0.03	1.12	Blank?
	AHI	0.04	1.03	
	Male gender	0.65	2.41	
	BMI (kg/m2)	0.964	1.00	
	Nocturnal BP dip	0.63	0.61	30%
2 (forward conditional)	desaturator	0.06	0.19	41%

10.6 Discussion

Age and AHI were significant predictors for the development of hypertension in OSAHS. In addition to these factors, male gender, BMI, and loss of nocturnal dip contribute to 30% of the total risk of hypertension in sleep apnoea patients. This suggests that hypoxaemia is a predictor of the development of hypertension in obstructive sleep apnoea. It explains 11% of the variance in hypertension, but this was statistically not significant ($p = 0.06$). It has been shown that the degree of nocturnal hypoxia, as a result of obstructive sleep apnoea, is more closely associated with the daytime level of blood pressure than with sleep fragmentation (Peled et al. 1998). In the same study, stepwise multiple regression analysis revealed that systolic BP was predicted by the combination of age and morning nor-epinephrine levels, whereas diastolic and mean BP were predicted by SaO2 alone or in combination with

age. The fact that minimal oxygen desaturation is a predictor for hypertension was reported from dog models when sustained daytime hypertension developed 2–5 weeks of induced apnoeas (Brooks et al. 1997). The mechanism behind the elevation of blood pressure as a result of hypoxia was thought to be due to tonic activation of chemoreflexes, which activates the sympathetic nervous system (Kara, Narkiewicz, & Somers 2003;Loredo et al. 2001;Narkiewicz et al. 1998c). In animal models, peripheral chemoreceptors were involved in the blood pressure response to chronic episodic hypoxia. Furthermore, chronic hypoxia with hypercapnia might cause greater increase in BP, similar to the OSAHS subjects, than hypocapnia hypoxia (Fletcher 2000). Activation of the renin-angiotensin system may also contribute to the elevation of blood pressure in chronic episodic hypoxia. Upregulation of the tissue angiotensin Π system appears to be necessary for the chronic blood pressure changes that occur from episodic hypoxia (Fletcher, Orolinova, & Bader 2001). In healthy subjects, interaction between chemoreceptor and baroreceptor reflexes caused by hypoxia and hypercapnia (Cooper et al. 2005), and this may contribute to the development of hypertension in OSAHS patients. Correction of intermittent nocturnal hypoxaemia by CPAP therapy might reduce blood pressure in OSAHS subjects as discussed before. However, a study by Norman et al. found that nocturnal supplemental oxygen did not affect blood pressure, although 2 weeks of CPAP therapy reduced both daytime and nighttime blood pressure (Norman et al. 2006). Thus CPAP may reduce BP by other mechanisms, such as reducing repetitive arousals in addition to abolishing the episodic hypoxaemia caused by the apnoea or hypopnoea (Okabe et al. 1995).

Chapter 11

Discussion

11.1 Baroreflex Study

In this thesis, the relationship between OSAHS and hypertension has been investigated. The initial discussion centred on the relationship between the two disorders from the epidemiological as well as some of the physiological aspects of the associations. The focus of the first study was to investigate the role of the baroreflex in the development and modulation of hypertension in patients with obstructive sleep apnoea/hypopnoea syndrome. The study was carefully designed to measure baroreflex activity during wakefulness without any interruption by an external stimulus such as light or sound, and no influences from ingested stimulants (caffeine, alcohol and smoking). The study showed no significant difference between the sequential domain measure of baroreceptor sensitivity or the spectral domains between the OSASH patients and controls. Furthermore, there was no change in BRS following one month of CPAP treatment.

In the intervention study, the patients improved symptomatically with CPAP, compared with the placebo (ESS, P = 0.02), but not with FOSQ (P = 0.3). FOSQ has been shown in one study to improve in OSAHS patients (Faccenda et al. 2001c) on CPAP and the lack of difference in this study might perhaps be explained by low power, although more severely affected patients were studied than those of Faccenda et al. in terms of desaturation (4% desaturation index was 18.8 in this study, compared with 7 in the Faccenda et al. study. Nevertheless, patients in this study were less symptomatic - the median ESS in this study was 13, compared with 15 in the Faccenda et al. study - which may explain the positive result in their study.

However, the study did not show any significant difference between CPAP and placebo treatment in the 48-hour BP measurement. This lack of difference was present when the data were analysed either on an-intention-to-treat basis or after sub-analysis by selecting the groups in whom greater changes might have been expected, the desaturating and compliant patients. Furthermore, there was no difference between CPAP and placebo when diurnal changes of BP data were analysed. The lack of difference in BP could perhaps be explained by the small sample size. Faccenda et al. found that there was a significant but marginal (1.4mmHg; P = 0.04) decrease in diastolic BP after 4 weeks of CPAP, compared with the placebo, with a sample size of 68 patients (Faccenda et al. 2001c). The CPAP-versus-placebo component of this study was only based on 29 patients, although 10 of them used their CPAP for more than 4 hours/night. Thus, a positive BP effect would not be expected with these small numbers.

The main focus of this study was the baroreflex sensitivity using non-invasive measures, and no significant difference was shown between the sequential domain measure or the spectral domains of baroreceptor sensitivity between the OSASH patients and controls. Furthermore, there were no changes in any measure of BRS with CPAP compared with the placebo. To the best of the writer's knowledge at the beginning of the study, this was the first trial that aimed to understand the role of baroreflex sensitivity in OSAHS during wakefulness in normotensive patients with a randomized trial of placebo and CPAP treatments. To understand the non-significant outcome of this treatment study, it is necessary to explore the methodological and physiological factors that perhaps might have contributed to the results.

11.1.1 Technical and Methodological Factors

The time limit of the study was determined by the four-weeks crossover study, for it has been shown that diastolic BP is reduced by CPAP over that period (Faccenda et al. 2001c). However, MSNA has been shown to decrease significantly after 6 months of CPAP treatment (Narkiewicz et al. 1999b). Other studies have used a similar technique in evaluating BRS in a before-and-after trial extending the CPAP treatment to three months (Bonsignore et al. 2002). Thus, a further study with an extended duration of therapy might be needed to determine whether there is any long term benefit. Although there have been studies that have measured the change in BRS after one night of CPAP treatment, they were small and not randomized (Logan et al. 2003;Tkacova et al. 2000). Tkacova et al. studied 8 OSAHS patients with congestive heart failure. CPAP increased the slope of BRS from 3.9 ms/mmHg pre CPAP to 6.2 ms/mmHg during one night of CPAP treatment. Bonsignore et al. found that three months of CPAP treatment increased BRS slope during nocturnal wakefulness ($P < 0.01$) and stage-2 sleep ($P = 0.0.5$) (Bonsignore et al. 2002). In a study by Logan at al., one night of CPAP treatment improved the slope of BRS ($P < 0.05$) in 11 OSAHS with refractory hypertension (see below).

Laboratory environment, such as noise in the corridor outside the bedroom, might have a stimulatory effect on patients. Every effort was made to minimize noise and the rooms were well soundproofed. Nevertheless, a change in the timing of the study might prevent this disturbance by avoiding the daily activities in the ward. Many studies examining the relationship between sleep apnoea and

baroreflex during sleep have been done (Mateika, Kavey, & Mitru 1999). A study by Logan (Logan et al. 2003) found that BRS slope improved while patients were using CPAP for one night. In this study, BRS was measured during stage-2 sleep to control for potential influences of the sleep state on the outcome variables. However, CPAP will alter lung volume while it is administered – but not in the daytime after CPAP use as in the current study – which could affect autonomic tone and thus BRS. Similarly Tkacova (Tkacova et al. 2000) studied OSAHS patients with congestive heart failure – not a pure OSAHS group – before and after CPAP treatment during stage-2 sleep for one night with a positive improvement in BRS slope while patients were on CPAP for 2–3 hours. However, this improvement did not last after CPAP, so the change in BRS was transitory. Furthermore, BRS was not been measured after CPAP when patients were fully awake during the morning, as in our study. In addition, the study was not well randomized and had a small sample size, as discussed below, and some heart failure patients were on beta blockers (Tkacova et al. 2000). Another study by Cooper et al,(Cooper et al. 2004) found in simulated obstructive sleep apnoea that breathing with inspiratory resistance reduces the sensitivity of the baroreceptor control of the vascular resistance. They also found that asphyxia produces an increased set point of the baroreceptor-vascular resistance reflex. However, they used neck chamber, which works by applying suction or pressure to the neck overlying the carotid sinuses.

Our sample size was relatively small which resulted in the low power of the study. Furthermore, in the sub-analysis of the compliant patients (CPAP use > 4

hour/night) and more hypoxic patients (4% DI > 10), the sample size became too small to show any significant difference (n = 9). The study was started by another researcher (Dr J. Faccenda), in which 13 OSAHS patients were included with no significant results either in whole or in part (13 + 29 = 42 patients). Most of the studies examining BRS in non-OSAHS populations used a larger sample power of more than a hundred subjects (Lantelme et al. 2002;Pinna et al. 2002). However significant changes in BRS with acute interventions have been reported in studies of samples of only 8 subjects (Tkacova et al. 2000) and 11 OSAHS patients with refractory hypertension (Logan et al. 2003). Bonsignore et al studied 29 normotensive OSAHS patients and 11 normal subjects, which is almost similar to our sample (Bonsignore et al. 2002).

Table 11.1 Sample Power calculation

	CPAP		Placebo		Least Mean difference detectable with 80% power
	Mean	S.D.	Mean	S.D.	
BRS (ms/mmHg)	4.9	2.5	5.5	4.4	2.6
VLFBRS(ms/mmHg)	3.1	1.3	3.5	2.3	1.4
LFBRS (ms/mmHg)	5.2	2.6	6.5	5.8	3.9
HFBRS (ms/mmHg)	8.7	6.5	9.5	9.3	6.0
AlphaBRS(ms/mmHg)	7.0	4.2	8.0	7.3	4.5

S.D. = standard deviation.

N = 29 patients

A two-tailed t-test was used for the power calculation, which was based on the data from our BRS study. From Table 12.1, it is clear that the sample size used in this study was small and larger numbers of patients are required to show any significant difference. In addition, it is now clear that the tests are very insensitive, particularly for the high frequency baroreflex (HFBRS), which is indicated by the higher mean difference required, eliciting a significant result. However, the study was time-consuming for staff and participants, and large resources would be needed to run the study with 80% power.

Validation of the methodology was probably the most important factor in the negative outcome of the study. The standard method of calculating BRS in the past was the change of pulse interval in response to infusion of phenylephrine (Floras et al. 1988). The sequence technique, a fairly new method, was introduced to replace the invasive method and it was validated in experimental animals (Bertinieri et al. 1988) and in humans (Parlow et al. 1995), though with different clinical conditions. It has been shown that there was a selective impairment of BRS in normotensive sleep apnoea patients during wakefulness, using the phenylephrine method (Carlson et al. 1996;Gerritsen et al. 2000;Mateika, Kavey, & Mitru 1999;Narkiewicz et al. 1998b). However, before this study was begun, there had been studies using the new technique of testing BRS, as discussed earlier, although in different settings and clinical conditions (Gerritsen et al. 2000;Mateika, Kavey, & Mitru 1999). At the beginning, our study was based on advice from the Professor David Webb's group, but we were not aware that the method was insufficiently validated.

Therefore, we undertook the validation reported here and found that the reproducibility of the tests was low. A separate study, at a later stage, demonstrated the lack of reproducibility of the spontaneous method of measuring BRS, both the sequence and, more importantly, spectral techniques. Spontaneous BRS measurement methods were very variable with time. This fluctuation may perhaps reflect baroreceptor physiology in addition to other factors, mainly respiration. Nevertheless, we also showed that spontaneous measurements were not a valid tool to assess baroreceptor sensitivity. It seems likely that this lack of reproducibility of the spontaneous baroreceptor gain is the main cause of the negative results of this study.

Patient selection. It could be argued that the patients who participated in our study did not have OSAHS that was severe enough (median AHI = 44.3 and 4%DI = 18.8). Bonsignore et al. found that CPAP improves baroreflex control of heart rate during sleep in OSAHS patients with mean AHI of 77 and SaO2 during NREM and REM of 81.2 and 67.7 respectively (Bonsignore et al. 2002).

Patient compliance was fair (median CPAP use = 4 hours/ night) which is good compared with other RCTs (Engleman & Douglas 1993b;Faccenda et al. 2001c) though perhaps not ideal. Pepperell et al. found that CPAP use of 5 hours/night reduced blood pressure (Pepperell et al. 2002) Compliance is often the main problem in CPAP use, and can be improved by intensive education and support programs (Hoy et al. 1999). In this study, patients were not only given standard care but were also contacted by me during each treatment to check for any problems.

11.1.2 Physiological Effect

1. Respiratory influences (see Chapter 7 on reproducibility). The human respiratory gate has a major influence in vagal and sympathetic control of the heart rate (Eckberg 2003). The heart rate interval (RRI) is very much under the influence of respiration by the respiratory cardiac coupling.

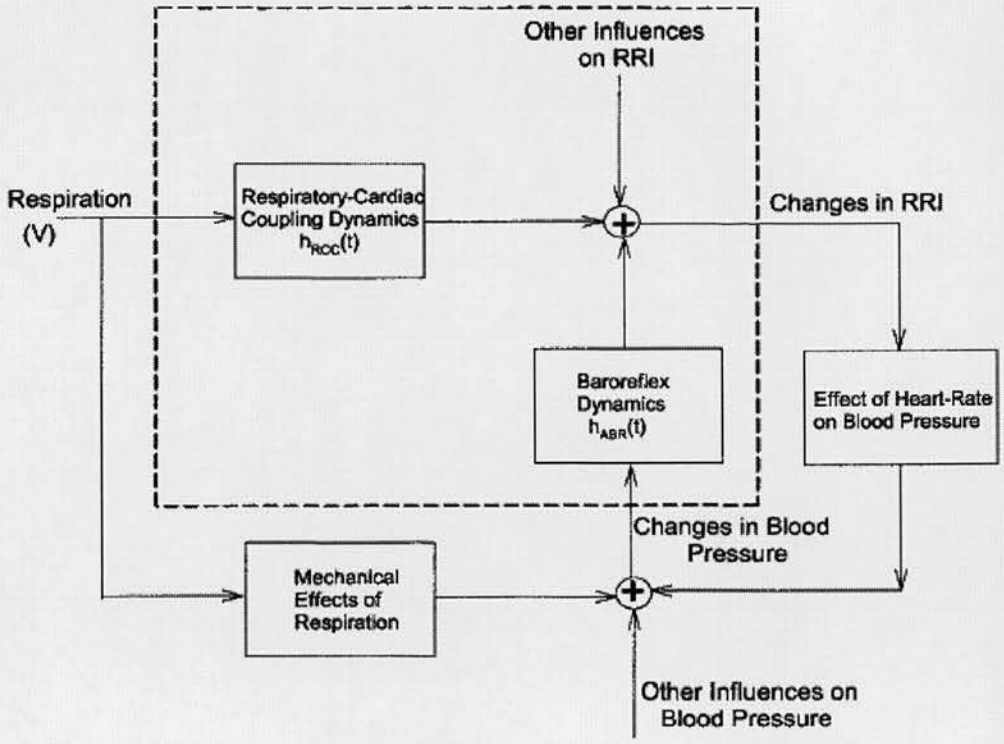


Figure 11.1 Schematic block diagram of the major physiological mechanisms that contribute to heart rate variability and blood pressure variability (Belozeroff, Berry, & Khoo 2003)

The most obvious marker of this respiratory influence is respiratory sinus arrhythmia. In this study, the patients were asked to breathe normally (tidal breathing). However, there was no control on patients to track the random breathing. This confounding respiratory factor can be accounted for if a mathematical model is used that relates changes in RRI to respiration and changes in systolic blood pressure. The model partitions the RRI fluctuation

into a component mediated by respiratory–cardiac coupling and one mediated by baroreflexes (Belozeroff, Berry, & Khoo 2003;Jo et al. 2003). It is non-invasive and a simpler means of quantifying the key aspects of autonomic control in spontaneously breathing OSAHS patients during wakefulness.

2. Age of the patients: BRS may diminish with age (see section 2.6.3). Furthermore, it has been shown, using different techniques, that age is negatively correlated with BRS (Lantelme et al. 2002). The decrease in baroreflex buffering with aging is related to increases in basal sympathetic nerve activity and reductions in systemic alpha1-adrenergic vascular responsiveness (Jones et al. 2003). The mean age of the patients in our study was 50 years. Nevertheless, there was no significant age effect ($P > 0.05$) in any parameter, although it is worth mentioning that the mean of BRS of patients above and below the age of 50 (the mean age) was 5.2 and 5.7 respectively. The rest of the measurements are in the table below.

Table 11.2 Age effect

	N	Mean	Std. Deviation
BRS (ms/mmHg)	12	5.2	2.41
	17	5.7	5.51
VLFBRS (ms/mmHg)	12	3.3	0.76
	17	3.7	2.98
LFBRS (ms/mmHg)	12	5.8	2.29
	17	6.9	7.36
HFBRS (ms/mm/Hg)	12	8.5	5.72
	17	10.1	11.29
Alpha (ms/mmHg)	12	7.2	3.70
	17	8.5	9.12

12 and 17 were the number of patients below and above the age of 50 respectively.

3. Diurnal changes. Normally, BRS increases at the transition from wakefulness to sleep (NREM sleep) and it decreases as subjects awake and resume their daily activities (Conway et al. 1983). Since in our study, all measurements were made at the same time of day, this cannot be a factor in our finding no change with CPAP. However, we cannot comment on changes at other times of the day.
4. Other physiological factors such as gender, neurohumeral and physical deconditioning (see section 2.6.3) might have a role in the outcome of this study. However, I do not have enough data in this study to comment further.

11.2 Vascular Endothelial Function Study

Venous occlusion plethysmography, coupled with brachial artery drug administration, has been widely used to study forearm blood flow (FBF) (Benjamin et al. 1995). It provides a safe and convenient arterial bed in which to test the vascular effects and endothelial function in sleep apnoea. In control arms (non-infused), the mean of forearm blood flow measurements was higher, though insignificant, which could be attributed to the difference in body size, since the severe OSAHS patients had higher BMI ($P = 0.03$) and neck and waist circumferences ($P = 0.03$ and $P = 0.04$ respectively). The non-infused arm is generally used as a contemporaneous control for the experimental arm, taking account of any minute-to-minute changes in blood flow that might affect both arms, such as those due to emotion or minor changes in the basal state (Benjamin et al. 1995). The data from the FBF measurement in the infused arm in the obstructive

sleep apnoea study found no significant difference between severe and mild OSAHS patients. This could be explained by the small sample size and thus low power, or genuine lack of difference. However, this study has not yet been completed and these patients represent only 25% of the projected number. In addition, the patients still need to be compared with matched healthy control subjects and also randomized for CPAP and sham CPAP in future.

Nevertheless, the study has its own limitations, for it is an invasive and technically demanding procedure, especially in overweight participants (mean BMI of the severe group was 38.6). Before this study was begun, there had been a few studies that had examined the endothelial function and OSAHS. An early study by Carlson et al. (Carlson, Rangemark, & Hedner 1996) indicated that endothelium-dependent vascular relaxation was attenuated in patients with sleep apnoea independently of hypertension. They used the FBF method to evaluate the endothelial function with acetylcholine and sodium nitroprusside infusion. A study by Kato et al. found that OSAHS patients had an impairment of resistance-vessel endothelium-dependent vasodilatation. However, they found no significant difference between sleep apnoea patients and control subjects, using the FBF method. In addition, endothelial function was assessed by measuring the brachial artery diameter, in which they found that there was a vascular response to sublingual administration of nitroglycerin (NTG) with a significant difference between sleep apnoea patients and control subjects (Kato et al. 2000). Nevertheless, the sample size of the study was small with 8 obstructive sleep apnoea patients and 9 healthy controls. In Kato et al's study, the patients had not been randomized to any treatment, and a non-invasive procedure of conduit-vessel dilatation was employed to obtain the difference. Another study was

conducted by Ip et al., in which the endothelial function was also evaluated by measuring the brachial artery diameter after sublingual nitroglycerin (Ip et al. 2004). In this study, the patients were randomized to receive CPAP treatment or no intervention. Ip et al. found that patients who received CPAP had a significant increase in flow-mediated dilatation. Our study was more comprehensive since it was a case-control study with different levels of severity. Furthermore, the more complex though standard procedure of plethysmography was employed in evaluating the endothelial effect of OSAHS with three different vasodilating drugs. Recently, it was found that CPAP therapy improved endothelial function in a non-randomised trial (Lattimore et al. 2006). They used similar methods to our study but a different drug protocol. They found that the response to Ach after 3 months of CPAP therapy was significantly greater compared to pre-CPAP response. N-monomethyl-L-arginine (L-NMMA) was co-infused with Ach 20μg. They also found no correlation between severity of nocturnal hypoxaemia and the magnitude of the change in vascular function with treatment. However, there was no comment about the correlation of the vascular function and hypoxaemia before the treatment.

11.3 Future Work

This thesis raises questions regarding the mechanisms of change in blood pressure in obstructive sleep apnoea. Further investigations are required to evaluate the autonomic nervous system and its relationship with the cardiovascular system as well as the endothelial function and growing understanding of the role of the endothelium in cardiovascular and cerebrovascular diseases in relation to OSAHS.

It would have been ideal if a better and validated methodology of the baroreflex sensitivity study had been used to test the baroreflex sensitivity in OSAHS with more

resources available. The reproducibility was the main problem in this study, which could be attributed to the factors previously discussed. In addition to the physiological and other methodological factors that have to be taken into consideration, the study would have been improved by having a higher power, longer period of time (for example, three months), and better control of confounding factors such as respiration. I would also suggest comparing the effect of CPAP during sleep and wakefulness to test the immediate and sustained effect of the treatment. This could also be tested with and without CPAP applied during wakefulness. More severe OSAHS patients should be included in any future work; who have more SaO2 desaturation and more symptomatic patients with higher ESS scores. However, recruiting hypertensive OSAHS patients might give a clearer picture about the relationship between obstructive sleep apnoea and hypertension and the role of the baroreflex (Floras et al. 1988;Lantelme et al. 2002;Siche et al. 1995). Nevertheless, it would be extremely difficult to recruit sufficient untreated hypertensive patients, thus it might be necessary to be less purist than in this study and include patients who are already on antihypertensive treatment. Because the relationship between sleep apnoea and hypertension is not confined to the baroreflex, other aspects should be explored. The role of the kidney should be investigated with more research on the effect of OSAHS on renal function (Fletcher 1993) and also the relationship between OSAHS and proteinuria. Although there have been a few studies that suggested an association between obstructive sleep apnoea and proteinuria, as discussed before, they were limited and none of them had been randomized, and they have produced divergent results. Earlier studies had shown that proteinuria is not uncommon in obese patients with OSAHS, which could be reversible (Sklar & Chaudhary

1988;Sklar, Chaudhary, & Harp 1989). However, more recent studies have reported that proteinuria is weakly associated with OSAHS (Casserly et al. 2001;Chaudhary, Rehman, & Brown 1995;Iliescu et al. 2001). Thus, a more comprehensive study is required to explore the role of the kidney in the development of hypertension in sleep apnoea patients. The study should be a case-control and randomized trial measuring renal function, BP, proteinuria with symptomatic assessment of urination habits.

The endothelium can greatly influence vascular tone and structure by releasing NO. Thus, the study of the endothelial function came with the mainstream of understanding the mechanism of development of hypertension and cardiovascular disorders in OSAHS. However, more studies are required to explore this issue, such as measuring free radicals or reactive oxygen species (Lavie 2003) that might be produced by hypoxia in patients with sleep apnoea. Furthermore, a few studies have shown increased expression of adhesion molecules (Lattimore et al. 2005) and production of reactive oxygen species in leukocytes of sleep apnoea patients (Dyugovskaya, Lavie, & Lavie 2002;Schulz et al. 2000). Pro-inflammatory factors such as interleukins, C-reactive protein, leukocyte adhesion molecules such as CD15 (Dyugovskaya, Lavie, & Lavie 2002) might also contribute to the pathogenesis of developing cardiovascular diseases, and merit further evaluation. In addition, prothrombotic factors (Robinson et al. 2004) such as fibrinogen, plasminogen activator inhibitor, and reduced fibrinolytic activity with enhanced platelet activity, may play a role in the process (Parish & Somers 2004). Thus, further randomized case-control studies would be of great value in understanding the pathogenesis of cardiovascular diseases in OSAHS. As I have discussed in chapter 9 there are other

methods of evaluating the endothelial function which could be used to evaluate the effect of CPAP therapy in OSAHS patients.

OSAHS has been shown to be associated with elevation of blood pressure. Much more work needs to be done to clarify the mechanisms for this association.

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Appendices

Appendix 1 EPWORTH SLEEPINESS SCALE

MW Johns. Sleep 1991, 14(6), 540-5.

Name: Study:

Date:

How likely are you to doze of or fall asleep in the following situations, in contrast to feeling just tired? This refers to your usual way if life in recent times. Even if you have not done some of these things try to work out how they would have affected you. Use the following scale to choose the most appropriate number for each situation.

0 = would never doze
1 = slight chance of dozing
2 = moderate chance of dozing
3 = high chance of dozing

SITUATION	Chance of dozing
Sitting reading	
Watching TV	
Sitting, inactive in a public place (e.g. a theatre or a meeting)	
As a passenger in a car for an hour without a break	
Lying down to rest in the afternoon when circumstances permit	
Sitting and talking to someone	
Sitting quietly after lunch without alcohol	
In a car, when stopped for a few minutes in the traffic	

Thank you for your co-operation

Appendix 2 FOSQ

Name: Date:

FUNCTIONAL OUTCOMES OF THE SLEEP QUESTIONNAIRE (FOSQ)

Some people have difficulty performing everyday activities when they feel tired or sleepy. The purpose of this questionnaire is to find out if you generally have difficulty carrying out certain activities because you are too sleepy or tired. In this questionnaire, when the words "sleepy" or "tired" are used, it means the feeling that you can't keep your eyes open, your head is droopy, that you want to "nod off", or that you feel the urge to take a nap. The words do not refer to the tired or fatigued feeling you may have after you have exercised.

DIRECTIONS: Please put a tick (3) in the box for your answer to each question. Select only one answer for each question. Please try to be as accurate as possible. All information will be kept confidential.

	(0) I don't do this activity for other reasons	(4) No difficulty	(3) Yes, a little difficulty	(2) Yes, moderate difficulty	(1) Yes, extreme difficulty
1 Do you have difficulty concentrating on the things you do because you are sleepy or tired?		☐	☐	☐	☐
2 Do you generally have difficulty remembering things, because you are sleepy or tired?		☐	☐	☐	☐
3 Do you have difficulty finishing a meal because you become sleepy or tired?		☐	☐	☐	☐
4 Do you have difficulty working	☐	☐	☐	☐	☐

on a hobby (for example, sewing, collecting, gardening) because you are sleepy or tired?

Functional Outcomes of Sleep questionnaire (FOSQ)

		(0) I don't do this activity for other reasons	(4) No difficulty	(3) Yes, a little difficulty	(2) Yes, moderate difficulty	(1) Yes, extreme difficulty
5	Do you have difficulty doing work around the house (for example, cleaning the house, doing laundry, taking out the rubbish, repair work) because you are sleepy or tired?	☐	☐	☐	☐	☐
6	Do you have difficulty operating a motor vehicle for short distances (less than 100 miles) because your sleepy or tired?	☐	☐	☐	☐	☐
7	Do you have difficulty operating a motor vehicle for long distances (greater than 100 miles) because your sleepy or tired?	☐	☐	☐	☐	☐
8	Do you have difficulty getting things done because you are too sleepy or tired to drive or take public transportation?	☐	☐	☐	☐	☐

9 Do you have difficulty taking care of financial affairs and doing paperwork (for example, writing cheques, paying bills, keeping financial records, filling out tax forms, etc) because you are sleepy or tired? ☐ ☐ ☐ ☐ ☐

10 Do you have difficulty performing paid or volunteer work because you are sleepy or tired? ☐ ☐ ☐ ☐ ☐

11 Do you have difficulty maintaining a telephone conversation because you become sleepy or tired? ☐ ☐ ☐ ☐ ☐

Functional Outcomes Of Sleep Questionnaire (FOSQ)

	(0) I don't do this activity for other reasons	(4) No difficulty	(3) Yes, a little difficulty	(2) Yes, moderate difficulty	(1) Yes, extreme difficulty
12 Do you have difficulty visiting with your family or friends in <u>your</u> home because you become sleepy or tired?	☐	☐	☐	☐	☐
13 Do you have difficulty visiting with your family or friends in <u>their</u> home because you become sleepy or tired?	☐	☐	☐	☐	☐

14 Do you have difficulty doing things for your family or friends because you are too sleepy or tired?

☐ ☐ ☐ ☐ ☐

	(4) No	(3) Yes, a little	(4) Yes, moderately	(5) Yes, extremely
15 Has your relationship with family, friends or work colleagues been affected because you are sleepy or tired?	☐	☐	☐	☐

In what way has your relationship been affected?

_____

_____

_____

_____

	(0) I don't do this activity for other reasons	(4) No difficulty	(3) Yes, a little difficulty	(2) Yes, moderate difficulty	(1) Yes, extreme difficulty
16 Do you have difficulty exercising or participating in a sporting activity because you are too sleepy or tired?	☐	☐	☐	☐	☐
17 Do you have difficulty watching a film or videotape because you become sleepy or tired?	☐	☐	☐	☐	☐

		(0) I don't do this activity for other reasons	(4) No difficulty	(3) Yes, a little difficulty	(2) Yes, moderate difficulty	(1) Yes, extreme difficulty
18	Do you have difficulty enjoying the theatre or a lecture because you become sleepy or tired?	☐	☐	☐	☐	☐
19	Do you have difficulty enjoying a concert because you become sleepy or tired?	☐	☐	☐	☐	☐
20	Do you have difficulty watching TV because you are sleepy or tired?	☐	☐	☐	☐	☐
21	Do you have difficulty participating in religious services, meetings or a group or a club because you are sleepy or tired?	☐	☐	☐	☐	☐
22	Do you have difficulty being as active as you want to be in the <u>evening</u> because you are sleepy or tired?		☐	☐	☐	☐
23	Do you have difficulty being as active as you want to be in the <u>morning</u> because you are sleepy or tired?		☐	☐	☐	☐

24 Do you have difficulty being as active is you want to be in the afternoon because you are sleepy or tired?

☐ ☐ ☐ ☐

24 Do you have difficulty keeping pace with others your own age because you are sleepy or tired?

☐ ☐ ☐ ☐

	(1) Very low	(2) Low	(3) Medium	(4) High
26 How would you rate your general level of activity?	☐	☐	☐	☐

Functional Outcomes of Sleep Questionnaire (FOSQ)

Appendix 3
Patient information sheet for the baroreflex sensitivity study

Patient Information Sheet

Blood pressure in sleep apnoea/hypopnoea study

The relationship between sleep apnoea and blood pressure remains controversial, we want to perform a study looking at the relationship between sleep apnoea and blood pressure, and also looking at the possible mechanism for the increases in blood pressure.

What does the study involve?

The study takes two months to complete, this is split into two 1-month blocks, and you will receive two different treatments, one for each month. We are using the standard treatment for sleep apnoea – CPAP (continuous positive airway pressure), and comparing this with a trial treatment. The second treatment involves taking an oral capsule once a day to see if it helps your symptoms and to see if it alters your blood pressure.

At the end of each month block we will measure your blood pressure for about 48 hours using a portable device which is worn around the waist. You will be asked to continue your normal activities whist wearing the device. Further recordings of blood pressure will be done in the morning with you lying down from your finger in a quiet room for approximately an hour while we also record your heartbeats.

I will also be asking you to fill out some questionnaires. I will also measure your weight and waist and neck size at each visit to the Sleep Centre and will take urine sample.

We will come out to your home to fit the blood pressure monitor at a convenient time for you and it will involve you coming up to the centre two days later for 1-2 hours in the morning, ideally before 10am. We will refund the travel costs for the extra two visits to the centre. At the end of the study you will be issued with a standard CPAP machine.

You are under no obligation to participate in this study and you can withdraw at any time without detriment to your future care.

All data collected will be confidential. Your General Practitioner will be informed of your participation in the study and of any abnormal results.

You can also contact Dr T.Mackay on 0131 536 1000 for unbiased advice on aspects of this study.

If you have any further questions please do not hesitate to get in touch, telephone 0131 536 4192/4196, or leave a message on 0131 536 2355.

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Clinical Research Fellow
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Specialist Registrar in Respiratory and General Medicine
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Appendix 4
patient information sheet for endothelial function
Patient Information Sheet

Title: THE EFFECT OF CPAP ON VASCULAR BIOLOGY IN THE OBSTRUCTIVE SLEEP APNOEA/HYPOPNOEA SYNDROME

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.
Consumers for Ethics in Research (CERES) published a leaflet entitled 'Medical Research and You'. This leaflet gives more information about medical research and looks at some questions you may want to ask. A copy may be obtained from CERES, PO Box 1365, London N16 0BW.
Thank you for reading this information sheet.

Patients with disordered breathing patterns during sleep have been shown to have increases in blood pressure that may have a bad effect on the heart and brain. This disordered breathing may result in detrimental effects on the blood vessels and lead to heart attacks and strokes. We seek to show that abnormal blood vessel responses are associated with low overnight oxygen levels and that treatment to assist breathing during sleep improves blood vessel function in people with low oxygen levels associated with their sleep apnoea. In addition we hope to look at a number of different genes and proteins to see if they can change the regulation of blood vessel responses. This will lead to an improvement in our understanding of such complex processes and the development of a new preventative treatment to reduce the risk of heart disease. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. You have been diagnosed with sleep apnoea and will be looked after and treated like any other patient that we see in our clinic. We will also ask to monitor your blood pressure using a special cuff over a period of 48 hours the two days before you have the test outlined below. The test itself consists of lying continuously on a bed for 2-3 hours in a warm room. During this time, some bands that will be placed around both arms will tighten up for short periods and from time to time will restrict your movements. A very small needle will be placed in the artery of one arm and two plastic tubes (cannulae) will be placed into the veins, one in each arm. This part of the study is associated with some slight discomfort and we use local anaesthetic to minimise this. The needle in the artery can occasionally become dislodged which would cause an ache and a slight bruise or swelling. If this does occur, the swelling usually resolves rapidly after stopping the study and removing the needle. You should, however, let us know if you experience any discomfort.

Small amount of drugs (substance P, acetylcholine and sodium nitroprusside) will be administered at very small that should only affect the arm. They can cause flushing and some mild swelling in the forearm. The effects are however self-limiting and of short duration.

Blood samples will be taken at intervals throughout the study and analysed for components of the blood that play a part in the clotting mechanism. The total amount of blood taken throughout the study will be less than 300 ml (or half a pint). Some blood samples may be retained to test for additional relevant proteins or genes, which could be important in determining the mechanisms under study. These samples will be destroyed at the end of the experiments.

.All information collected about you during the course of the research, including blood samples and test results, will be kept strictly confidential. Any information about you that leaves the hospital will have your name and address removed so that you cannot be recognised from it. We will inform your General Practitioner of your participation in this study.

If taking part in this research project harms you, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available to you.

The Lothian Medicine/Clinical Oncology Research Ethics Sub-Committee has reviewed the study.

You will be given a copy of the information sheet and a signed consent form to keep. Please do not hesitate to ask the investigators any questions and you may also ask for advice from the Local Independent Advisor, Dr Thomas Mackay, who is not directly involved with the study.

Once again, thank you for your attention.

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Consent form for the OSAHS and baroreflex study

LOTHIAN RESEARCH ETHICS COMMITTEE

STANDARD CONSENT FORM

TITLE OF THE PROPOSED RESEARCH:

Hypertension in Sleep Apnoea/Hypopnoea Syndrome.

NAME OF INVESTIGATOR:

Dr M Alabri / Dr J. F. Faccenda

ADDRESS:

Sleep Centre, Ward 48, Royal Infirmary of Edinburgh.

TELEPHONE:

0131 536 2355

FURTHER INFORMATION IS AVAILABLE FROM: (A person who is not involved in the study)

Dr T Mackay

LIST ANY DRUGS TO BE GIVEN IN THE STUDY EXPLAINING THEIR ACTION:

Trial capsule to improve upper airway tone.

LIST ANY PROCEDURES REQUIRED IN ADDITION TO THE STANDARD PROCEDURES:

48 hour blood pressure monitoring, beat by beat blood pressure

- **I agree to participate/to the patient/subject participating* in this study.**
- **I have read this consent form and Patient/Subject Information Sheet and had the opportunity to ask questions about them.**
- **I agree for notice to be sent to my/the patient's/subject's* General Practitioner about my/their* participation in this study.**
- **I agree to the provision of any clinically significant information to my/the patient's/subject's General Practitioner.**
- **I understand that I am/the patient/subject is* under no obligation to take part in this study and that a decision not to participate will not alter the treatment that I/the patient/subject* would normally receive.**
- **I understand that I have/the patient/subject has* the right to withdraw from this study at any stage and that to do so will not affect my/their* treatment.**
- **I understand that this is non-therapeutic research from which I/the patient/subject* cannot expect to derive any benefit.***